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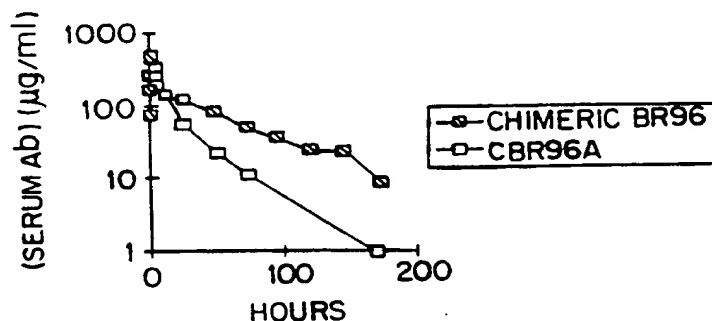
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(54) Title: A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS

**(57) Abstract**

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an immunoglobulin or Ig fusion protein molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by inactivation of at least a portion of the constant region.

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5 **A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED
TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN
THERAPY AND IN VIVO DIAGNOSIS**

10 Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

15 **TECHNICAL FIELD OF THE INVENTION**

The present invention relates to methods for inhibiting or reducing immunoglobulin-induced toxicity resulting from therapy or in vivo diagnosis. Specifically, in lieu of using unmodified antibodies or recombinant binding proteins for in vivo use, the
20 invention provides the use of modified antibodies or recombinant binding proteins which have been structurally altered in the constant domain so that upon administration immunoglobulin-induced toxicity is reduced or inhibited.

BACKGROUND OF THE INVENTION

25

Over the years investigators have attempted to harness the immune system for therapeutic use. Immunoglobulin (Ig) molecules which constitute an important part of the immune system are of great interest because they (1) react with a diverse family of ligands, (2) possess different effector functions and (3) are of great
30 biological importance. Despite its potential, a persistent problem with

immunoglobulin immunotherapy has been, among other problems, the toxic effect to normal cells of using antibodies which recognize both normal and diseased cells. This problem is far-reaching because the majority of antibodies presently available recognize a target located on both normal and diseased cells (Slavin-Chiorini, et al.,
5 Int. J. Cancer 53: 97-103 (1993)).

The constant region can promote cell death through antibody dependent cell mediated cytotoxicity (ADCC) or by complement dependent cytotoxicity (CDC). Despite the deletion of portions of the constant region, particularly the CH₂ domain,
10 the antigen binding function can be retained (D. Yelton, M. Scharf, Mutant monoclonal antibody with alterations in biological functions, J. Exp. Methods 156:1131-1148 (1982)).

Others have generated a CH₂-deleted antibody (Mueller et al., Proc. Natl. Acad. Sci.
15 USA 87: 5702-5705 (1990)). Their findings provide that the CH₂-deleted antibody was cleared from the blood of tumor-bearing mice much faster than the corresponding intact antibody. Other in vivo findings also confirmed that a CH₂-deleted antibody, designated ch14.18DCH2, is a potentially useful reagent for radioimmunodetection of human tumors because of its reduced immunogenicity,
20 increased target specificity, and rapid clearance from circulation (Mueller et al., Proc. Natl. Acad. Sci. USA 87: 5702-5705 (1990)).

Generally, whole antibody molecules are composed of two heavy (H) and two light (L) chains which are held together by covalent bonds (disulfide) and non-covalent
25 interactions. Each chain contains a variable region (V) and a constant region (C). The variable regions at the amino termini of the two chains form the antigen binding region. The constant region of the H chain has three components or domains. Occasionally, the first constant region domain (CH₁) interacts with the C region of the L chain through hydrophobic interactions and generally a disulfide bond,

depending on isotype. The next C region stretch is the hinge-acting disulfide bond stably introduced between two H chains. The second constant region domain (CH₂) is adjacent to the hinge region. CH₂ contains sequences important for effector functions of the antibody, such as the sequences responsible for complement
5 fixation, and Fc receptor binding. The third constant region domain (CH₃) is located at the carboxyl terminus of the H chain, and is considered to play an important role in H chain assembly as well as some C region functions.

Today many antibodies in clinical trials are directed against tumor associated
10 antigens. Most tumor associated antigens are not tumor specific but are also generally found on the cell surface of some normal, non-tumorigenic cells. The clinical use of some antibodies directed against tumor associated antigens are limited because of the toxicity associated with their use. Therefore, there is a need for methods for inhibiting toxicity associated with immunoglobulin use in the field of
15 disease therapy (e.g., therapy for tumors, kidney disease, and the like) and in vivo diagnosis.

We addressed this need by discovering methods for inhibiting or reducing toxicity to normal cells generally associated with immunoglobulin immunotherapy or in vivo
20 diagnosis, wherein the immunoglobulin recognizes both diseased and normal cells. Our discovery involves generating immunoglobulin molecules or Ig fusion proteins having structurally altered constant regions which inhibit or reduce immunoglobulin-induced toxicity.

25 SUMMARY OF THE INVENTION

The present invention provides methods for inhibiting immunoglobulin-induced toxicity by using known immunoglobulin or Ig fusion protein molecules which are structurally altered in their constant regions so that the resulting structurally altered

immunoglobulin or Ig fusion protein molecules exhibit reduced or inhibited toxicity in vivo compared to their original unmodified counterparts.

Structural alteration of the constant region may be effected in a number of ways as long as it results in reducing or inhibiting immunoglobulin-induced toxicity.

In accordance with the practice of the invention, structural alteration of the constant region is effected by deletion of the entire constant region. In another embodiment, only the CH₂ domain is deleted. In another embodiment, only that portion of the CH₂ domain that binds the Fc receptor is deleted. In yet another embodiment, only that portion of the CH₂ domain that binds the complement component C1q is deleted. Alternatively, in another embodiment, multiple deletions in discrete Fc receptor and complement component binding domains are effected.

Alternatively, structural alteration is effected by single or multiple mutations in the CH₂ domain such as amino acid insertions and substitutions. The mutation or mutations must result in inhibiting immunoglobulin-induced toxicity. By way of example, the amino acids in multiple toxicity associated domains in the constant region can be altered so as to render the constant region unable to mediate a ADCC response or activate complement thereby inhibiting immunoglobulin induced toxicity resulting from immunotherapy. Alternatively, multiple amino acids in a single toxicity associated domain in the constant region can be altered.

Further alternatively, structural alteration can be effected by isotype switching resulting in an altered immunoglobulin molecule that either does not induce toxicity or induces some limited toxicity but does not cause a harmful effect. For example, isotype switching can result in the constant region being unable to mediate a CDC or ADCC response or some other activity which mediates toxicity.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a line graph showing plasma clearance in high Le^y expressing dogs using chimeric BR96 versus constant region mutant of cBR96-2.

5

Figure 2 is a schematic diagram of a plasmid designated pTWD-cJVK.L1 including the chimeric (c)BR96-light chain (SEQ ID NO. 11).

Figure 3 is a schematic diagram of a plasmid designated pD16hJ1.L1 including the
10 human (h)BR96-light chain (SEQ ID NO. 13).

Figure 4 is a schematic diagram of a plasmid, designated pD17-hJm14-dCH2.H1, of hBR96-2A (i.e., human mutant BR96 having the H1, H2, and H3 mutations and the CH₂ deletion (PCT Application No. 95/305444, published March 6, 1996)).

15

Figure 5 is a schematic diagram of a plasmid, designated pD17-cJ-dCH2.H1, of cBR96-A (SEQ ID NO. 10) (i.e., chimeric BR96 having the CH₂ deletion (PCT Application No. 95/305444, published March 6, 1996)).

20 Figure 6 is a schematic diagram of a plasmid, designated pD17-cJ.H1, of cBR96.

Figure 7 is a line graph showing the results of an ELISA assay of (1) hBR96-2A-Dox to Le^y (closed diamond), (2) hBR96-2A to Le^y (96:0006A2 R/A)(closed square), (3) hBR96-2A to Le^y (96:0006B R/A)(closed triangle), and BR96-Dox to
25 Le^y (X).

Figure 8 is a line graph showing the results of an ELISA assay of (1) BR96-A-Dox to Le^y (closed diamond), (2) chiBR96 to Le^y (closed square), (3) cBR96-A to Le^y (96:0003 R/A)(closed triangle), and cBR96-Dox to Le^y (X).

Figures 9a-c are schematic diagrams showing the steps for deleting a CH₂ domain.

Figures 10a-c are schematic diagrams showing the construction of BR96 IgG1 CH₂
5 domain point mutations.

Figure 11 is a schematic diagram showing the construction of the pNg1/14 vector.

Figure 12 is a schematic diagram showing the construction of pD17-hBR96-2.
10

Figure 13 is a schematic diagram showing the construction of pD17-hJm14-
dCH2.H1.

Figure 14 is the nucleic acid sequence of pD17-cJ-dCH2.H1, the plasmid shown in
15 Figure 5, chimeric BR96 having the CH₂ deletion.

Figure 15 is a line graph showing the results of an ELISA assay comparing whole
chiBR96 and deleted CH₂ chiBR96 on Le^y.

20 Figure 16 is a description of the seven structural alterations.

Figure 17 is a schematic diagram of a plasmid designated pD17-hG1b.

Figure 18 is the nucleic acid sequence of pD17-hJm14.H1.
25

Figure 19 is the nucleic acid sequence of pD17-hG1b.

Figure 20 is a line graph showing complement dependent cytotoxicity. In the
legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is

hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b mAb, negative control.

- 5 Figure 21 is a line graph showing antibody dependent cell-mediated cytotoxicity. In the legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b monoclonal antibody (mAb), negative control.

10

Figure 22 is a line graph showing binding activity of hBR96-2 constant region mutants on LeY-HSA. In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331
15 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

- Figure 23 is a line graph showing binding activity of hBR96-2 constant region mutants on LNFPIII-BSA. LNFPIII is a lacto-N-fucopentasose, a Lewis X
20 trisaccharide with an additional lactose spacer (V Labs, Covington, LA). In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

25

Figures 24A and 24B provide a strategy for introducing multiple mutations by RPCR. (A) Diagram of the 1.4 kpb IgG heavy chain region showing the hinge CH₂ and CH₃ domains as boxed regions. Site-specific mutations to be introduced into CH₂ positions L1, L2, and L3 are encoded by complementary sets of mutant PCR

primers (A1 and A2; B1 and B2; and C1 and C2). The asterisks (*) indicate the number of amino acid changes introduced at each L position. The two PCR primers, Rs (Recombination -sense) and Ra (Recombination-antisense), flank the Eco-47-III restriction sites and mediate homologous recombination with vector ends. The 3' ends of the oligonucleotides are represented by arrowheads. (B) A three-way homologous recombination event between fragments RsA2, A1Ra and the linearized vector produces the L1 mutant IgG. Two distally located sets of mutations (L1 and L2) are simultaneously introduced by increasing the number of recombining PCR products as is shown in the four-way recombination of RsA2, A1B1, B1Ra with vector.

Figure 25 is a gel showing Eco-47-III restriction endonuclease analysis of DNAs prepared from colonies generated by multiple PCR fragment RPCR. Lane M: 1kb ladder DNA marker (GIBCO/BRL Life Science Technology). Lanes 1-12: Twelve randomly selected colonies resulting from quadruple homologous recombination events were used to prepare plasmid and digested with Eco47-III. Clones 1, 2, 6 and 9 contain the fully assembled 1.4 kpb insert.

Figure 26 provides the amino acid sequence for hBR96-2 heavy-chain variable region and the human IgG1 constant region.

Figure 27 provides the amino acid sequence for hBR96-2A heavy-chain variable region and the human IgG1 constant region.

Figure 28 provides the amino acid sequence for chi BR96 heavy-chain variable region and the human IgG1 constant region without the CH₂ domain.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

5 As used herein the term "inhibiting immunoglobulin-induced toxicity" means to reduce or alleviate symptoms generally associated with toxicity caused by immunoglobulin or Ig fusion protein therapy, e.g., toxicity mediated by effector functions of the Fc receptor. For example, BR96 antibody recognizes and binds BR96 antigen which is found at some levels in the gastrointestinal tract and at
10 elevated levels in tumors (as compared to the gastrointestinal tract of normal tissues). The binding of BR96 antibody to BR96 antigen in vivo causes symptoms associated with gastrointestinal toxicity. These symptoms include rapid onset of vomiting, often with blood, and nausea. In humans the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach.

15 The pathology of the wound is limited and resolves. However, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity. For highly elevated levels of other antigens found in the central nervous system (CNS), liver, and other locations, the toxicity will be characterized by
20 symptoms other than those described above.

As used herein the term "immunoglobulin molecule" can be produced by B cells or be generated through recombinant engineering or chemical synthetic means. Examples of immunoglobulin molecules include (1) antibodies, e.g., polyclonal and
25 monoclonal antibodies, chimeric or humanized, and (2) recombinant Ig containing binding proteins, e.g., Ig fusion proteins. Recombinant Ig containing binding proteins include cell surface proteins, e.g., CD antigens (in one embodiment, CTLA4), to which an Ig tail is joined.

As used herein the terms "structurally altered" or "structural alteration" means manipulating the constant region so that the resulting molecule or protein exhibits a diminished ability to induce toxicity. Structural alteration can be by chemical modification, proteolytic alteration, or by recombinant genetic means. Recombinant
5 genetic means may include, but is not limited to, the deletion, insertion and substitution of amino acid moieties.

As used herein the terms "multiple toxicity associated domains" means more than one discrete toxicity associated domain. As there appear to be at least two toxicity
10 associated domains in the immunoglobulin molecule, one roughly localized to amino acids 231-238 and another roughly localized to amino acids 310-331, an example of the structural alteration of multiple toxicity associated domains comprises the insertion, substitution or deletion of amino acid residues in both of these domains. This definition excludes structural alterations targeting a single toxicity associated
15 domain.

Merely by way of example, the constant region of the immunoglobulin molecule can be structurally altered so that the molecule no longer mediates a CDC or ADCC response. However, the methods of the invention encompasses the use of
20 structurally altered immunoglobulin molecules regardless of whether it mediates a CDC or ADCC response. The underlying requirement is that the altered molecule must inhibit immunoglobulin-induced toxicity.

Structural alteration can be effected in a number of ways. For example, structural
25 alteration can be effected by deletion of the entire constant region.

Alternatively, structural alteration can be effected by deletion of the entire CH₂ domain of the constant region. In this instance, deletion of the entire CH₂ domain may render the molecule unable to (1) bind an Fc receptor thereby eliminating the

molecule's possibility of mediating antibody-dependent cellular cytotoxicity (ADCC), (2) bind C1q, or (3) activate complement.

Alternatively, structural alteration can be effected by deletion of only that portion of
5 the CH₂ domain that binds the Fc receptor or complement.

Further alternatively, a single mutation or multiple mutations such as substitutions and insertions in the CH₂ domain can be made. The underlying requirement of any mutation is that it must inhibit, diminish, or block immunoglobulin-induced toxicity.
10 For example, this can be achieved by mutating the constant region such that the altered molecule is rendered unable to mediate a CDC response or an ADCC response, or to activate complement.

Alternatively, structural alteration can be effected by isotype switching (also known
15 as class switching) so that the altered molecule does not induce toxicity in the subject. In one embodiment, the constant region of the immunoglobulin is structurally altered so that it no longer binds the Fc receptor or a complement component, e.g., switching a molecule's original IgG isotype from IgG1 to IgG4. Isotype switching can be effected regardless of species, i.e., an isotype from a non-
20 human being can be switched with an isotype from a human being (E.D. Finkelman et al. (1990) Annu. Rev. Immunol. 8:303-333; T. Honjo et al. (1979) Cell 18: 559-568; T. Honjo et al. In "Immunoglobulin Genes" pp. 124-149 Academic Press, London)).

25 As used herein the term "Ig fusion protein" means any recombinantly produced antigen or ligand binding domain having a constant region which can be structurally altered.

As used herein "cytotoxic agent" includes antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents. Specific examples within these groups include but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, 5 tenoposide, vincristine, vinblastine, colchicine, supporin, gelonin, PE40, bryodin, dihydroxy anthracin dione, actinomycin D, and 1-dehydrotestosterone.

As used herein the term "BR96" refers to (1) the whole BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, (2) chimeric BR96 10 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, or (3) BR96 mutant molecules disclosed in PCT No. 95/305444, published March 6, 1996.

As used herein, "treating" means to (1) provide tumor regression so that the tumor is 15 not palpable for a period of time (standard tumor measurement procedures may be followed (A.B. Miller et al. "Reporting results of cancer treatment" Cancer 47:207-214 (1981)); (2) stabilize the disease; or (3) provide any clinically beneficial effects.

As used herein, an "effective amount" is an amount of the antibody, 20 immunoconjugate, or recombinant molecule which kills cells or inhibits the proliferation thereof.

As used herein, "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular or 25 subcutaneous administration, or the implantation of a slow-release device such as a miniosmotic pump, to the subject.

As used herein, "pharmaceutically acceptable carrier" includes any material which when combined with the antibody retains the antibody's specificity or efficacy and is

non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including
5 coated tablets and capsules.

Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may
10 also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

As used herein, "mutation" means a single amino acid or nucleic acid mutation or multiple mutations by whatever means, e.g., homologous recombination, error prone
15 PCR, or site directed mutagenesis.

In order that the invention herein described may be more fully understood, the following description is set forth.

20 METHODS OF THE PRESENT INVENTION

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from the use of immunoglobulin during therapy or in vivo diagnosis. For example, the methods of the invention would be useful to minimize
25 the toxicity associated with prolonged clinical exposure to immunoglobulin use during or after tumor imaging with radiolabeled antibodies.

In accordance with the practice of this invention, the subject includes, but is not limited to, human, equine, porcine, bovine, murine, canine, feline, and avian

subjects. Other warm blooded animals are also included in this invention.

This method comprises administering an immunoglobulin molecule to the subject. The immunoglobulin can be IgG, IgM, or IgA. IgG is preferred.

5

In one embodiment of the invention, the immunoglobulin molecule recognizes and binds Le^y. In another embodiment, the immunoglobulin recognizes and binds Le^x. In a further embodiment, the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma deposited on February 22, 1989 with the American Type
10 Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and
accorded ATCC Accession No.: HB 10036. In yet another embodiment, the
immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma
deposited on May 23, 1990, with the ATCC, 12301 Parklawn Drive, Rockville, MD
20852 and accorded ATCC Accession No.: HB 10460.

15

In accordance with the practice of the invention, the immunoglobulin can be a
bispecific antibody with a binding specificity for two different antigens, one of the
antigens being that with which the monoclonal antibody BR96 produced by the
hybridoma having the identifying characteristics of HB 10036 as deposited with the
20 ATCC binds. Also, in accordance with the practice of the invention, the
immunoglobulin can be an anti-idiotypic antibody.

25

As required by the invention, at least a portion of the constant region of the
immunoglobulin molecule is structurally altered. Structural alteration can be
effected by a number of means. In one embodiment, the entire constant region, i.e.,
CH₁, CH₂, and CH₃ domains, can be deleted.

In another embodiment, only the CH₂ domain is deleted from the immunoglobulin
molecule (e.g., cBR96-A (Figure 5), hBR96-2A (Figure 4). In this embodiment, the

CH₂ deletion may result in a molecule unable to bind the Fc γ receptor or a complement component.

In another embodiment, only that portion of the CH₂ domain which binds the complement component C1q is deleted. In yet another embodiment, mutations in specific portions of the CH₂ domain are made. For example, the immunoglobulin molecule may be modified by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited. A discussion of such mutations are further found hereinafter.

10

Regardless of the means, the underlying requirement for any structural alteration of the constant region is that immunoglobulin-induced toxicity is substantially reduced or inhibited. In one embodiment, immunoglobulin-induced toxicity is inhibited by structurally altering the constant region such that the molecule's ability to mediate a CDC response or ADCC response and/or activate the complement cascade is prevented or inhibited. Methods for determining whether the molecule is able to inhibit a CDC response are well known, e.g., one method involves a ⁵¹Cr-release test (H. Garrigues et al. Int. J. Cancer 29:511 (1982); I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to inhibit an ADCC response are well known (I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to activate a complement cascade are well known.

In another embodiment of the invention, the method comprises administering to the subject an Ig fusion protein having a structurally altered constant region. Structural alteration of the constant region may include deletion of the entire C region or portions thereof, e.g., alteration of the CH₂ domain so that the altered molecule no longer binds the Fc receptor or a complement component.

The invention further provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject. The method comprises administering to the subject an antibody which has been modified so that at least a portion of the constant region has been structurally altered as discussed supra. In one
5 embodiment, the antibody recognizes and binds Le^y. In another embodiment, the antibody recognizes and binds to Le^x.

In accordance with the practice of this invention, the antibody can be monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of
10 HB 10036 as deposited with the ATCC. Alternatively, the antibody can be chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC. Further, the antibody can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma
15 having the identifying characteristics of HB 10036 as deposited with the ATCC binds.

Additionally, the present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a
20 subject. The disease will vary with the antigen sought to be bound. Examples of diseases include but are not limited to immunological diseases, cancer, cardiovascular diseases, neurological diseases, dermatological diseases or kidney disease.

25 This method comprises the following steps. Step one provides selecting an antibody for a target. Generally, the target is associated with the disease and the antibody directed to the target is known. For example, the target can be the BR96 antigen and the antibody selected is BR96.

Step two of this method provides structurally altering the constant region of the antibody so selected so that immunoglobulin induced toxicity is inhibited. Inactivation can include any of the means discussed above. For example, inactivation can be effected by structurally altering multiple toxicity associated
5 domains in the CH₂ domain of the constant region of the Ig protein so selected.

Step three of this method provides administering the structurally altered antibody of step two to the subject under conditions that the structurally altered antibody recognizes and binds the target and that such binding directly or indirectly alleviates
10 symptoms associated with the disease.

In accordance with the invention, in one embodiment step one provides selecting an Ig fusion protein for a target. Further, the method provides mutating the Ig fusion protein so selected by structurally altering the CH₂ domain of the constant region of
15 the Ig protein by the same means discussed above.

The invention further provides methods to treat human carcinoma. For example, the immunoglobulin, antibody, or Ig fusion protein discussed above can be used in combination with standard or conventional treatment methods such as
20 chemotherapy, radiation therapy or can be conjugated or linked to a therapeutic drug, or toxin, as well as to a lymphokine or a tumor-inhibitory growth factor, for delivery of the therapeutic agent to the site of the carcinoma.

Techniques for conjugating therapeutic agents to immunoglobulins are well known
25 (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellström et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In

Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)).

5

Alternatively, the structurally altered antibody or Ig fusion protein can be coupled to high-energy radiative agents, e.g., a radioisotope such as ^{131}I ; which, when localized at the tumor site, results in a killing of several cell diameters (see, e.g., Order, "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985)). According to yet another embodiment, the structurally altered BR96 antibody can be conjugated to a second antibody to form an antibody heteroconjugate for the treatment of tumor cells as described by Segal in United States Patent 4,676,980.

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Still other therapeutic applications for the structurally altered antibody or Ig fusion protein of the invention include conjugation or linkage, e.g., by recombinant DNA techniques or protein chemical techniques, to an enzyme capable of converting a prodrug into a cytotoxic drug and the use of that antibody-enzyme conjugate in combination with the prodrug to convert the prodrug to a cytotoxic agent at the tumor site (see, e.g., Senter et al., "Anti-Tumor Effects Of Antibody-alkaline Phosphatase", Proc. Natl. Acad. Sci. USA, 85:4842-46 (1988); "Enhancement of the in vitro and in vivo Antitumor Activities of Phosphorylated Mitomycin C and Etoposide Derivatives by Monoclonal Antibody-Alkaline Phosphatase Conjugates", Cancer Research 49:5789-5792 (1989); and Senter, "Activation of Prodrugs by Antibody-Enzyme Conjugates: A New Approach to Cancer Therapy," FASEB J. 4:188-193 (1990)).

20
25

It is apparent therefore that the present invention encompasses pharmaceutical compositions including immunoglobulin molecules, antibodies, and Ig fusion proteins all having structurally altered CH₂ domains, and their use in methods for treating human carcinomas. For example, the invention includes pharmaceutical
5 compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of a structurally altered BR96 and a pharmaceutically acceptable carrier.

The compositions may contain the structurally altered antibody or Ig fusion protein
10 or antibody fragments, either unmodified, conjugated to a therapeutic agent (e.g., drug, toxin, enzyme or second antibody). The compositions may additionally include other antibodies or conjugates for treating carcinomas (e.g., an antibody cocktail).

The compositions of the invention can be administered using conventional modes of
15 administration including, but not limited to, intrathecal, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

The composition of the invention can be in a variety of dosage forms which include,
20 but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

25 The compositions of the invention also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

In accordance with the practice of the invention, the pharmaceutical carrier can be a lipid carrier. The lipid carrier can be a phospholipid. Further, the lipid carrier can be a fatty acid. Also, the lipid carrier can be a detergent. As used herein, a detergent
5 is any substance that alters the surface tension of a liquid, generally lowering it.

In one example of the invention, the detergent can be a nonionic detergent. Examples of nonionic detergents include, but are not limited to, polysorbate 80 (also known as Tween 80 or (polyoxyethylenesorbitan monooleate), Brij, and Triton (for
10 example Triton WR-1339 and Triton A-20).

Alternatively, the detergent can be an ionic detergent. An example of an ionic detergent includes, but is not limited to, alkyltrimethylammonium bromide.

15 Additionally, in accordance with the invention, the lipid carrier can be a liposome. As used in this application, a "liposome" is any membrane bound vesicle which contains any molecules of the invention or combinations thereof.

The most effective mode of administration and dosage regimen for the compositions
20 of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician.

The interrelationship of dosages for animals of various sizes and species and humans based on mg/m^2 of surface area is described by Freireich, E.J., et al. Cancer
25 Chemother., Rep. 50 (4): 219-244 (1966). Adjustments in the dosage regimen can be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses can be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided doses can be

administered daily or proportionally reduced depending on the specific therapeutic situation).

THE MOLECULES OF THE INVENTION

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The present invention provides structurally altered BR96 or BR96 Ig fusion proteins. Structurally altered BR96 antibodies or Ig fusion proteins have the variable region of BR96 and a modified constant region. This modification provides structurally altered BR96 antibodies or Ig fusion proteins with the ability to inhibit immunoglobulin-induced toxicity.

10

Various embodiments of structurally altered BR96 or BR96 Ig fusion proteins have been made.

15 In one embodiment, designated cBR96-A, the entire CH₂ domain of cBR96 was deleted. CBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 10. cBR96 is expressed by a plasmid having the sequence in SEQ ID NO. 9.

20 In another embodiment, designated hBR96-2A, the entire CH₂ domain of hBR96 was deleted. hBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 12. hBR96 is a mutant BR96 having the H1, H2, and H3 mutations described in PCT Application No. 95/305444, published March 6, 1996.

25 In yet another embodiment, designated hBR96-2B, the leucine residue located at amino acid position 235 is mutated to alanine. Additionally, the glycine residue located at amino acid position 237 is mutated to alanine. The amino acid position numbering used is described in Kabat et al. Sequences of Proteins of Immunological Interest 5th Edition (1991) United States Department of Health and Human Services.

In a further embodiment, designated hBR96-2C, the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine
5 using standard protocols (Alexander R. Duncan and Greg Winter "The binding site for C1q on IgG" Nature 332:738 (1988)).

In another embodiment, designated hBR96-2D, the proline residue at position 331 is mutated to alanine (M-H. Tao et al., "Structural features of human immunoglobulin
10 G that determine isotype-specific differences in complement activation" J. Exp. Med. 178:661-667 (1993); Y. Xu et al., "Residue at position 331 in the IgG1 and IgG4 domains contributes to their differential ability to bind and activate complement" J. Biol. Chem. 269:3469-3474 (1994)).

15 In an additional embodiment, designated hBR96-2E, the leucine residue at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine (A. Morgan et al., "The N-terminal end
20 of the CH₂ domain of chimeric human IgG1 anti-HLA-DR is necessary for C1q, Fc(gamma)RI and Fc(gamma)RIII binding" Immunol. 86:319-324 (1995)).

In yet a further embodiment, designated hBR96-2F, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is
25 mutated to alanine; and the proline residue located at position 331 is mutated to alanine.

In yet another embodiment, designated hBR96-2G, the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is

mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

5 In another embodiment, designated hBR96-2H, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

10

Depending on its form, a structurally altered BR96 antibody or fusion protein can be a monofunctional antibody, such as a monoclonal antibody, or bifunctional antibody, such as a bispecific antibody or a heteroantibody. The uses of structurally altered BR96, i.e., as a therapeutic or diagnostic agent, will determine the different forms of
15 structurally altered BR96 which is made.

Several options exists for antibody expression. Immunoexpression libraries can be combined with transfectoma technology, i.e., the genes for the Fab molecules derived from the immunoglobulin gene expression library can be connected to the
20 desired constant-domain exons. These recombinant genes can then be transfected and expressed in a transfectoma that would secrete an antibody molecule.

Once produced, the polypeptides of the invention can be modified, i.e., by amino acid modifications within the molecule, so as to produce derivative molecules. Such
25 derivative molecules would retain the functional property of the polypeptide, namely, the molecule having such substitutions will still permit the binding of the polypeptide to the BR96 antigen or portions thereof.

It is a well-established principle of protein chemistry that certain amino acid

substitutions, entitled "conservative amino acid substitutions." can frequently be made in a protein without altering either the conformation or the function of the protein.

- 5 Amino acid substitutions include, but are not necessarily limited to, amino acid substitutions known in the art as "conservative".

Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid
10 (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa.

Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional
15 structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine and valine (V).

Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R)
20 are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

- 25 In one embodiment of the present invention, the polypeptide is substantially pure, i.e., free of other amino acid residues which would inhibit or diminish binding of the polypeptide to its target and would inhibit or reduce gastrointestinal toxicity which are normally exhibited during or after antibody therapy.

NUCLEIC ACID MOLECULES ENCODING THE PRESENT INVENTION

The nucleotide sequences and the amino acid sequences of the variable and constant regions of BR96 are known. The sequence for the immunoglobulin constant region
5 is known and provided in Figure 18. Specific mutations in the constant region of the BR96 antibody were made. Nucleic acid molecules encoding the seven mutants described above (hBR96-2B through hBR96-2H) are as follows.

In hBR96-2B, alanine at amino acid positions 235 and 237 is encoded by codons
10 GCU, GCC, GCA, or GCG.

In hBR96-2C, serine at positions 318, 320, and 322 is encoded by UCU, UCC, UCA, or UGG.

15 In hBR96-2D, alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2E, alanine at positions 235 and 237 is encoded by codons GCU, GCC, GCA, or GCG. Serine at positions 318, 320, and 322 is encoded by UCU, UCC,
20 UCA, or UGG.

In hBR96-2F, alanine at positions 235, 237, and 331 is encoded by codons GCU, GCC, GCA, or GCG.

25 In hBR96-2G, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG. Further, the alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2H, alanine at positions 235, 237, and 331 is encoded by codons GCU,

GCC, GCA, or GCG. Additionally, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG.

Any of the above can be deoxyribonucleic acid (DNA), e.g., complementary DNA
5 (cDNA), or ribonucleic acid (RNA).

IMMUNOCONJUGATES

Immunoconjugates (having whole antibody or Ig fusion proteins) may be
10 constructed using a wide variety of chemotherapeutic agents such as folic acid and anthracyclines (Peterson et al., "Transport And Storage Of Anthracyclines In Experimental Systems And Human Leukemia", in Anthracycline Antibiotics In Cancer Therapy, Muggia et al. (Eds.), p. 132 (Martinus Nijhoff Publishers (1982); Smyth et al., "Specific Targeting of Chlorambucil to Tumors With the Use of
15 Monoclonal Antibodies", J. Natl. Cancer Inst., 76:503-510 (1986)), including doxorubicin (DOX) (Yang and Reisfeld "Doxorubicin Conjugated with a Monoclonal Antibody Directed to a Human Melanoma-Associated Proteoglycan Suppresses Growth of Established Tumor xenografts in Nude Mice PNAS (USA)" 85:1189-1193 (1988)), Daunomycin (Arnon and Sela "In Vitro and in vivo Efficacy
20 of Conjugates of Daunomycin With Anti-Tumor Antibodies" Immunol. Rev., 65:5-27 (1982)), and morpholinodoxorubicin (Mueller et al., "Antibody Conjugates With Morpholinodoxorubicin and Acid-Cleavable Linkers", Bioconjugate Chem., 1:325-330 (1990)).

25 BR96 has been conjugated to doxorubicin and has been shown to be effective in therapy of certain cancers or carcinomas (Trail, P.A., Willner, D., Lasch, S.J., Henderson, A.J., Casazza, A.M., Firestone, R.A., Hellström, I., and Hellström, K.E. Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. Science, 261:212-215, 1993).

In accordance with the practice of the invention, structurally altered BR96 can be used in forms including unreduced IgG, reduced structurally altered IgG, and fusion proteins (PCT Application No. 95/305444, published March 6, 1996).

5

Suitable therapeutic agents for use in making the immunoconjugate includes Pseudomonas exotoxin A (PE) in either the native PE or LysPE40 form. LysPE40 is a truncated form containing a genetically modified amino terminus that includes a lysine residue for conjugation purposes. Doxorubicin is also a suitable therapeutic agent.

10

Additional examples of therapeutic agents include, but are not limited to, antimetabolites, alkylating agents, anthracyclines, antibiotics, and anti-mitotic agents.

15

Antimetabolites include methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine.

20

Alkylating agents include mechlorethamine, thiotepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin.

25

Anthracyclines include daunorubicin (formerly daunomycin) and doxorubicin (also referred to herein as adriamycin). Additional examples include mitozantrone and bisantrene.

Antibiotics include dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC).

Antimitotic agents include vincristine and vinblastine (which are commonly referred to as vinca alkaloids).

- 5 Other cytotoxic agents include procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons.

Further examples of cytotoxic agents include, but are not limited to, ricin, bryodin, gelonin, supporin, doxorubicin, taxol, cytochalasin B, gramicidin D, ethidium
10 bromide, etoposide, tenoposide, colchicine, dihydroxy anthracin dione, 1-dehydrotestosterone, and glucocorticoid.

Clearly analogs and homologs of such therapeutic and cytotoxic agents are encompassed by the present invention. For example, the chemotherapeutic agent
15 aminopterin has a correlative improved analog namely methotrexate.

Further, the improved analog of doxorubicin is an Fe-chelate. Also, the improved analog for 1-methylnitrosourea is lomustine. Further, the improved analog of vinblastine is vincristine. Also, the improved analog of mechlorethamine is
20 cyclophosphamide.

METHODS FOR MAKING MOLECULES OF THE INVENTION

There are multiple approaches to making site specific mutations in the CH₂ domain
25 of an immunoglobulin molecule. One approach entails PCR amplification of the CH₂ domain with the mutations followed by homologous recombination of the mutated CH₂ into the vector containing the desired immunoglobulin, e.g., hBR96-2. For example, hBR96-2B and hBR96-2D have been made by this method.

Another approach would be to introduce mutations by site-directed mutagenesis of single-stranded DNA. For example, vector pD17-hG1b, which contains only the constant region of IgG1 and not the V domain of hBR96, has the fl origin of replication. This gives the vector the properties of a phagemid and site-directed mutagenesis experiments can be performed according to the methods of Kunkel, et al. (Kunkel, T.A., J.D. Roberts, and R.A. Zakour, 1987 Methods Enzymol. 154:367-383) as provided in the Bio-Rad Muta-Gene® phagemid *in vitro* mutagenesis kit, version 2. For example, hBR96-2B, -C, -D, -E, -F, -G, and -H were made by this method.

10

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the scope of this invention in any manner.

15

EXAMPLE 1

The following standard ELISA protocol was used.

- 20 **Materials:** Immulon2 96 well plates and Genetic Systems Specimen Diluent Concentrate (10x); antibody conjugate was Goat Anti Human Kappa-HRP Mouse Adsorbed, Southern Biotech. at 1:10,000 in Genetic Systems Conjugate Diluent (1x); Genetic Systems EIA Chromogen Reagent (TMB) (1:100); Genetic Systems EIA Buffered Substrate (1x); primary antibody or antigen were AffiniPure F(ab')₂ Fragment Goat Anti Human IgG Fc Fragment specific (Jackson Immuno Research),
25 Goat Anti Human Kappa-UNLB (Southern Biotechnology Associates), Le^y-HSA (Alberta Research Council).

Methods: Dilute primary antibody or antigen to 1.0 µg/ml in 0.05M Carb/Bicarb buffer. Add 100µl of the diluted solution per well in Immulon 2 plates. Seal plates and incubate O.N. at 4°C.

- 5 Block plates by flicking them and blotting on paper towels. Add 200µl/well of Genetic Systems, Specimen Diluent Concentrate (1x). Incubate at least 1 hour at room temperature and then dump the contents of the plates. Wash the plates 3x in saline/Tween. Blot to dry. Allow the plates to dry at R.T. (45 min. to 1 hour). Seal and store the plates at 4°C.

10

Test samples as follows. Dilute samples and standards in Specimen Diluent at 1:10. Perform serial dilutions in separate round bottom plates. Transfer 100µl/well of final dilutions to antigen coated assay plates; then incubate O.N. at 4°C. Wash plates 3x with saline/Tween.

15

For conjugation add 100 µl/well of antibody-HRP conjugate in Genetic Systems Conjugate Diluent (1x). Incubate plates at Room Temp. for 60 min. Wash plates 3x in saline/Tween.

- 20 Add 100 µl/well of Genetic Systems EIA Chromogen Reagent (TMB) 1:100 in EIA Buffered Substrate (1x). Incubate at R.T. for 15 min. and stop with 1N H₂SO₄ 100 µl/well. Read plate at 450/630nm in EIA plate reader.

EXAMPLE 2

25

Construction of CH₂ deleted BR96 molecules

Strategy for Deleting CH₂ Domains: To construct CH₂ deleted BR96 molecules, the hinge, CH₂ and CH₃ domains were removed from chimeric BR96 and humanized

BR9696-2 IgG1 molecules by an Eco47-III restriction digestion in non-coding regions. The hinge and CH₃ domains were amplified by polymerase chain reaction (PCR) from a human IgG1 (pN γ 1.14) molecule lacking the CH₂ domain. Two oligonucleotides (Sense 49mer, Antisense 50mer) homologous to the sequences of
5 IgG1 constant region at both sides preserving E.co47-III sites were synthesized. The amplified hinge and CH₃ domain PCR fragments were added into Eco47-III sites on BR96 IgG1 molecules by in vivo homologous recombination (P. Bubeck et al., Nucleic Acid Research (1993) 21:3601-3602). The new BR96 IgG1 molecules were verified by restriction mapping and sequencing.

10

A sewing PCR strategy was used for the construction of CH₂ deleted human IgG1 (pN γ 1.14) (Robert M. Horton, et al. (1990) Biotech 8 (5)P, 528).

The CH₁ domain was amplified as a 580 bp fragment with a sense oligonucleotide
15 (5' TGG CAC CGA **AAG CTT** TCT GGG GCA GGC CAG GCC TGA 3') (primer A) and an antisense oligonucleotide (5' **TCC GAG CAT GTT GGT ACC CAC GTG GTG GTC GAC** GCT GAG CCT GGC TTC GAG CAG ACA 3') (primer B) from a linearized human IgG1 constant region vector (pN γ 1.7). The PCR fragment extends from the 5' end of the Hind-III site (in bold) through the Cel-II, Sal-I, Dra-
20 III, Kpn-I, 6 bp nucleotide spacer and Mro-I sites (in bold) at the 3' end of the CH₁ domain.

The CH₃ domain was then partially amplified (to the Xba-I site) with a sense primer
(5' **GTC GAC CAC CAC GTG GGT ACC AAC ATG TCC GGA** GCC ACA
25 TGG ACA GAG GCC GGC T 3') (primer C) and an antisense primer (5' CTG GTT CTT GTT CAT CTC CTC **TCT AGA** TGG 3') (primer D) from a linearized human IgG1 constant region vector (pN γ 1.7). A PCR fragment (about 150 bp) with Sal-I, Dra-III, Kpn-I, 6 nucleotide spacer and Mro-I sites (in bold) on its 5' end, extends only through the Xba-I site (in bold) within the CH₃ domain.

The CH₁ and CH₃ partial PCR fragments were combined in a PCR without any primer. The reaction was run through two full cycles of denaturation and re-annealing to allow the fragments to combine at the homologous region at the 3' ends. Primers A and D (described above) were added to the reaction and the PCR cycle was completed. The polymerase extends the DNA with primer A and primer D, yielding a full-length (660 bp) PCR fragment. The newly extended PCR fragment is arranged from the 5' end to the 3' end in the following order: Hind-III - CH₁ - Cel-II - Sal-I - Dra-III - Kpn-I - 6 bp spacer - Mro-I - CH₃ partial - Xba-I.

The combined PCR fragment, with the CH₁ and partial CH₃ domains, was then cloned by a blunt end ligation into a Sma-I site on a pEMBL18 vector and the sequence was confirmed by dideoxy sequencing (Sanger et al. (1977) PNAS (USA) 74:5463-5466).

To transfer the CH₁ and partial CH₃ into a mammalian expression vector, both the pEMBL18 and pNγ1.7 vectors were digested with Hind-III and Xba-I. The Hind-III and Xba-I fragment was ligated into the same sites on a linearized pNγ1.7 vector. The new construct, with CH₁ and a full CH₃ domain, was designated the pNγ1.10 vector.

The hinge fragment was amplified from a Hind-III digested pNγ1.7 vector with the primers designed to flank the hinge exon with a Sal-I and a Dra-III cloning site at each end. These sites also exist between the CH₁ and CH₃ domains of the pNγ1.10 construct. The sense oligonucleotide (5' ACC ATG **GTC GAC** CTC AGA CCT GCC AAG AGC CAT ATC 3') with a 6 bp spacer and a Sal-I cloning site (in bold) and the antisense oligonucleotide (5' CAT GGT **CAC GTG** GTG TGT CCC TGG ATG CAG GCT ACT CTA G 3') with a 6 bp spacer and a Dra-III cloning site (in bold) were used for the amplification of the hinge fragment (250 bp).

The hinge region PCR fragment was cloned into a Sma-I site on pEMBL18 by blunt end ligation. Both the pEMBL18 with the hinge domain and the pN γ 1.10 with the CH₂ and CH₃ domains were digested with Sal-I and Dra-III. The digested hinge
5 fragment was cloned into the Sal-I and Dra-III linearized sites on the pN γ 1.10 vector. The new construct, now carrying the CH₁, hinge and CH₃ domains, was designated pN γ 1.11.

To make the final CH₂ deleted human IgG1 construct, both the pN γ 1.11 construct
10 and pN γ 1.11 vector were digested with BamHI and HindIII. A fragment containing the CH₁, hinge and CH₃ domains was cloned into the linearized pN γ 1.11 vector. The new constant region IgG1 construct lacks the CH₂ domain and is designated pN γ 1.14 (Figure 11).

15 For digestion of BR96 IgG1 with Eco47-III, a restriction fragment with hinge, CH₂ and CH₃ domains was identified on the constant region sequence of BR96 IgG1 vector in both chimeric and humanized molecules. The 5' end of this fragment lies inside the intron between CH₁ and hinge and the 3' end is located inside the CH₃ intron of the BR96 IgG1 molecule. The hinge, CH₂ and CH₃ domains (1.368 kb
20 fragment) were removed from BR96 IgG1 molecules by Eco47-III restriction digestion. The Eco47-III is a blunt end cutter. The BR96 IgG1 DNA digested with this enzyme does not require any pretreatment before cloning. Figure 12 is a diagrammatic representation of the pD17-hBR96-2 vector showing the Eco47-III sites used in cloning.

25

The CH₂ deleted BR96 IgG1 was then constructed as follows. The hinge and CH₃ domains were amplified from a CH₂ deleted L6 IgG1 (pN γ 1.14) construct with a sense oligonucleotide (5'

CAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAG**CGCT**GACCTCAG

- A 3') homologous to the constant region sequence of IgG1 at the 5' end of the Eco47-III site (in bold) and an antisense oligonucleotide (5'GGAAAGAACCATCACAGTCTCGCAGGGG CCCAGGGCAGCGCTGGGTGCTT 3') homologous to the constant region
- 5 sequence of IgG1 at the 3' end of the Eco47-III site (in bold). The Eco47-III site at the 3' end of the pN γ 1.14 construct is modified in the cloning process. The Eco47-III site is thus introduced into an antisense primer and used in amplification of the hinge and CH₃ domains.
- 10 The pD17-BR96 IgG1 vector was digested with Eco47-III and the hinge, CH₂ and CH₃ domains were removed. The linearized pD17-BR96 IgG1 vector was mixed with equimolar amounts of hinge and CH₃ PCR fragments. Cotransformation of the PCR fragment with linearized DNA into E.coli DH5a competent cells resulted in a recombinant molecule, mediated by homologous recombination in bacteria. This
- 15 construct lacks the CH₂ domain of BR96 IgG1 molecules, and is designated pD17-BR96-dCH2 (Figure 13).

1.9 grams of CH₂-deleted chimeric BR96 was obtained as raw material from 89L of culture supernatant.

20

EXAMPLE 3

Toxicity, localization and clearance of CH₂-deleted chimeric BR96 was tested in vivo as follows.

25

Three dogs received 400 mg/m² of cBR96-A, the CH₂ deletion mutant of chimeric BR96, and two received chimeric BR96. Both molecules had been mildly reduced and alkylated. This is required to prevent dimerization of the deletion mutant into a tetravalent form. Both control dogs experienced the typical GI toxicity and none of

the three receiving the mutant displayed any toxicity. The control dogs and two of the test dogs were sacrificed at 1 hr to obtain duodenal tissue to measure antibody localization. Both control dogs had grossly visible GI pathology, and the test dogs had normal appearing GI tissue. The third dog has continued to show no signs of toxicity.

Results: A significant amount of localization of the CH₂ deleted cBR96 (cBR96-A) occurred to the GI tract in dogs treated with 400 mg/m², although the intact chiBR96 localized slightly better. The levels of localization indicate that roughly equivalent amounts of intact and CH₂ deleted cBR96 was delivered to the GI tract in these dogs.

Table 5. Localization of cBR96 to GI tissue.

Group	Animal	Specific	mean
Localization			
cBR96	#271	155	135
	#272	114	
cBR96-A	#273	126	89
	#274	52	

15

Using the mean level of specific localization, an amount of cBR96-A equivalent to at least 66% of the amount of cBR96 was delivered to the target organ of toxicity, the duodenum. Based on the dose ranging done with cBR96 in dogs (some clinical signs of toxicity seen at doses of 10 mg/m²), even if this difference is real, it could

not explain the difference between significant toxicity and no toxicity, evaluation to date indicated that dogs treated with cBR96-A had no toxicity, pending microscopic histopathologic examination. This evaluation was based on analysis of 2 frozen blocks per dog and 2 sections per block. Replicates were quite good. We also ran
5 historical frozen tissues from dogs treated with native cBR96 or F(ab)₂/BR96 and the levels of localization for those tissues were 110 and 0, respectively, consistent with our previous data.

Assuming that there is no toxicity at marginally higher (2X) doses of cBR96-A,
10 these data indicate that the CH₂ domain is associated with the induction of acute gastroenteropathy, and that the removal of this domain prevents the induction of gastroenteropathy mediated by BR96.

This study confirms the results showing that F(ab')₂ is not toxic in the dog model
15 and that the toxicity is mediated by the constant region. The CH₂ deletion mutant is a candidate for targeting agents clinically. Because of the very long half-life of chimeric BR96, some decrease in the mutant's half-life should be acceptable.

Figure 1 shows the measurement of the clearance of the cBR96-A in high Le^y
20 expressing dogs. The study used chimeric versus constant region mutant of cBR96-2.

CBR96-2 did clear faster than the chimeric BR96. The localization of cBR96-A to the gastrointestinal epithelium is not significantly affected by this more rapid
25 clearance. More than enough of the cBR96-A localized to have caused toxicity.

Discussion: The constant region of chimeric IgG is responsible for the GI toxicity seen in clinical trials, e.g. with chiBR96-dox. The GI toxicity seen in the dog model is very similar to the clinical toxicity. Both in man and dog, administration of the

unconjugated antibody mediates an acute GI toxicity characterized by rapid onset of vomiting, often with blood.

5 In man the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach. Although the pathology of the wound is limited and resolves, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity.

10 This toxicity is mediated in man and dog by the antibody molecule alone. At higher doses of the antibody-dox conjugate, additional toxicity is seen in the dog model, probably due to doxorubicin. Although the intact IgG of BR96 causes toxicity in dog and man, the F(ab')₂ molecule (divalent and lacking only in the constant region) is not toxic in dogs. This finding has motivated our attempts at high levels, and improves the affinity and specificity of BR96 for tumor antigen.

15

The CH₂ domain is known to mediate complement and FcR binding. It was not known that structural alteration of the CH₂ domain would result in immunoglobulin-induced toxicity inhibition.

20 Toxicology study of hBR96-2B

The toxicology study of hBR96-2B in high Lewis Y expressor dogs (n=2) showed that a dose of 400 mg/m² did not cause hematemesis nor bloody stools, in contrast to BR96 which consistently causes one or both signs. A dog sacrificed at 24 hrs had
25 normal gross appearance of the GI tract, again in marked contrast to chimeric BR96 which causes hemorrhagic lesions and mucosal erosions.

EXAMPLE 4

- The polymerase chain reaction (PCR) is a widely used and versatile technique for the amplification and subsequent modification of immunoglobulin genes. The
- 5 rapidity and accuracy with which antibody genes can be modified in vitro has produced an assortment of novel antibody genes can be modified in vitro has produced an assortment of novel antibodies. For example, PCR methods have been used for engineering antibodies with increased affinity to antigen, for "humanizing" antibodies, and for modulating effector function (Marks, J.D., A.D. Griffiths, M.
- 10 Malmqvist, T. Clackson, J.M. Bye and G. Winter. 1992. Bypassing immunization: high affinity human antibodies by chain shuffling. *Bio/Technology* 10:779-783; Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96
- 15 Fab. *J. Biol. Chem.* 271:22611-22618; Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. *Immunology.* 86:319-324).
- 20 As part of a more comprehensive study, we desired to introduce various site specific mutations in the CH₂ constant domain of human IgG₁. Six specific amino acid residues distributed throughout the CH2 domain previously identified to play a role in immune effector function were marked as targets for mutagenesis (Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-
- 25 terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. *Immunology.* 86:319-324; Duncan, A.R. and G. Winter. 1988. The binding site for C1q on IgG. *Nature* 332:738-740; Tao, M.-H., R.I.F. Smith and S.L. Morrison. 1993. Structural features of human immunoglobulin G that determine isotype-specific differences in complement

activation. J.Exp.Med. 178:661-667). five of the six residues were grouped into two clusters-one cluster consisting of two residues, two amino acids apart (Location 1, or L1); and a second cluster consisting of three residues spanning a sequence of five amino acids (L2). The remaining amino acid position (L3) made for the total of six
5 residues. We were interested in constructing a panel of mutant CH₂ domain IgGs consisting of each L mutation by itself as well as in combination with other L mutants (e.g., L1; L1; and L2; L1, L2 and L3; etc.).

Various *in vitro* methods have been described where PCR is used to simultaneously
10 introduce distally located site-specific mutations within a gene sequence (Ho, S.N., H.D. Hunt, R.M. Horton, J.K. Pullen and L.R. Pease. 1989. Site-directed mutagenesis by overlap extension. Gene 77:51-59; Ge, L. and P. Rudolph. 1996. Simultaneous introduction of multiple mutations using overlap extension PCR. BioTechniques 22:28-30). Alternatively, an *in vivo* procedure termed recombination
15 PCR (RPCR) has also successfully been used for rapidly and efficiently generating distally located site-specific mutations (Jones, D.H. and S.C. Winistorfer. 1993. Use of polymerase chain reaction for making recombinant constructs. p.241-250. In B.A. White (Ed.), Methods in Molecular Biology, Vol. 15. Humana Press Inc., Totowa, NJ, Jones, D.H. And B.H. Howard. 1991. A rapid method for
20 recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. BioTechniques 10:62-66). RPCR uses *E. Coli*'s recombination machinery to generate intact circular recombinant plasmids from a transfected mixture of linear PCR-generated product and linearized vector. *In vivo* recombination is mediated through the joining of nucleotide sequences designed into
25 the 5' ends of both PCR primers that are homologous to DNA sequences encoded by the vector. In this report we describe an extension of the RPCR procedure for simultaneously introducing complex combinations of mutations into an antibody CH₂ domain.

Humanized BR96 variable region heavy and light chain genes, previously cloned and co-expressed as an assembled active Fab fragment in an M13 phage expression vector, provided the starting material (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. *J. Biol. Chem.* 271:22611-22618). The heavy and light chain V genes were amplified by PCR from a single-stranded M13 DNA template and subcloned by *in vivo* recombination (Jones, D.H. And B.H. Howard. 1991. A rapid method for recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. *BioTechniques* 10:62-66) into vectors pD17-hG1a and pD16-hC κ , to form pBR96-hG1a and pBR96-hC κ respectively. pD17-hG1a and pD16-hC κ are eukaryotic immunoglobulin expression vectors derived from pcDNA3 (Invitrogen, San Diego, CA). The plasmid pBR96-hG1a was further modified by site-directed mutagenesis to introduce two Eco47-III restriction sites flanking the immunoglobulin hinge-CH₂-CH₃ domains using standard procedures. The recipient vector was then prepared by digesting pBR96-hG1a with Eco47-III, isolating the vector backbone by agarose gel electrophoresis followed by extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA).

20

The strategy for introducing multiple mutations within the immunoglobulin CH₂ gene, shown in Figure 24, relies on the *in vivo* homologous recombination of several independently amplified PCR products with each other as well as with the pBR96-hG1a vector DNA. For introducing mutations at two distal locations two PCR products are synthesized (Figure 24B). One end of each PCR product is for recombining with an homologous end of the linear vector, and the other end, encoding the mutation(s) of interest, is for recombining with the neighboring PCR product. As shown in Figure 24B, additional distally-located mutations can be introduced into a target sequence by increasing the number of PCR products

25

proportionately. The recombination of neighboring PCR products always occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends (e.g., A1, A2) contain complementary mutant residues.

The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers (Rs and Ra) contain sequences for recombining with the end regions of the Eco47-III digested pBR96-hG1a vector.

Each L mutation was amplified in a separate PCR reaction. The reaction conditions were 250 ng intact pBR96-hG1a DNA template, 10 ul of 1X *Pfu* buffer (Stratagene, Inc. San Diego, CA), 10 nmol dNTPs, 200ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase in a 100ul reaction volume. Samples were first denatured at 95° C for 5 min, cooled to 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, followed by a final extension at 72°C for 7 min in a Perkin-Elmer DNA Thermal Cycler (Norwalk, CT). The amplified products were purified from a 1% agarose gel, extracted with Qiagen Gel Extraction kit and the recovered DNA quantitated. 50 ng of each PCR product was mixed with 25 ng of the Eco47-III digested pBR96-hG1a vector, transfected into Max competent *E. coli* DH5α according to the manufacturer's procedure (GIBCO BRL/Life Technologies, Gaithersburg, MD), and the entire transfection reaction plated onto selective LB agar plates containing 100 ug/ml ampicillin.

25

The results of several cloning experiments are summarized in the Table that follows. Typically the transformations produced from 80 to 200 bacterial colonies. Individual colonies were selected and grown overnight in 2 ml liquid cultures for isolation of miniprep plasmid DNA (Qiagen) and analysis by Eco47-III restriction

endonuclease mapping. Among 24 independent transformants analyzed from triple homologous recombination events (two PCR products plus vector) 11 clones contained the predicted 1.4 kpb DNA insert.

- 5 Figure 25 shows a sample diagnostic restriction analysis of DNA prepared from clones derived from quadruple homologous recombination events (three PCR products plus vector). Additional sampling of clones resulting from quadruple recombination yielded a cloning efficiency of 29% (7 clones containing inserts/24 clones sampled). At this point, due to the small sampling sizes, we do not know
10 whether the differences in the cloning efficiencies observed between the triple and quadruple recombination events are meaningful.

- To evaluate the expression of Le γ -binding activity of the CH₂ mutant IgGs, miniprep DNAs from 6 clones derived from the triple recombination reaction and 6
15 clones derived from the quadruple recombination reaction exhibiting the predicted diagnostic Eco47-III restriction patterns were isolated, mixed with pBR96- hCk DNA and used to co-transfect COS7 cells. 48 hour spent supernatants from 3 ml cultures were assayed for total IgG production and for Le γ binding activity by enzyme-linked immunosorbent assay (EIA) as described (Yelton, D.E., M.J. Rosok,
20 G.A. Cruz, W.L. Cosand, J. Bajorath, I. Hellstom, K.-E. Hellstorm, W.D. Huse and S.M. Glaser. 1995. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. J.Immunol. 155:1994-2004). All twelve cultures were found to secrete approximately 2-3 ug/ml Le γ -reactive IgG. The spectrum of Le γ binding activities were all similar to that of native humanized BR96 IgG indicating
25 that the homologously recombined antibodies did not acquire any gross mutations that could affect antigen binding. To confirm that the desired CH₂ mutations had been incorporated, and to evaluate the recombined genes for misincorporated nucleotides, four of the clones producing functional antibody were sequenced using Sequenase Version 2 DNA Sequencing Kit (United States Biochemical). One clone

was found to contain a single nucleotide change within the forward PCR primer used for mediating recombination with vector DNA. We are uncertain whether this error occurred during chemical synthesis of the oligonucleotide primer or is a result of misincorporation during the PCR reaction, despite the fact that we used a

5 thermostable polymerase with proofreading activity.

A RPCR procedure for homologously recombining up to three separate PCR-generated mutated antibody sequence products into a eukaryotic expression vector for the rapid construction of engineered IgG molecules is described herein. The

10 advantage of this approach is the ability to simultaneously introduce multiple distally-located mutations with PCR products synthesized by a single round of PCR. Recombinant DNAs are produced with a reasonably high cloning efficiency and fidelity of correct nucleotide sequences. The ability to efficiently rejoin several distinct PCR products should permit combinatorial strategies for constructing

15 complexly mutated protein domains as well as broadening the number and location of desired mutations.

Analysis of transformants generated by multiple-fragment RPCR.

Mutant IgGs Constructed	PCR Fragments in reaction	HR ^a events	Colonies Analyzed	Cloning Efficiency ^b
2	2	triple	24	45%
2	3	quadruple	24	33%
^a HR-homologous recombination				
^b Cloning efficiency (number of clones containing 1.4kbp insert/total number of colonies)				

EXAMPLE 5

This example provides two methods for introducing site specific mutations into the
5 CH2 domain of human IgG1 constant region containing vectors.

One method involves PCR amplification of a segment or segments of the constant
region, wherein mutations are introduced using appropriately constructed
oligonucleotides. The vector receiving the fragment(s) is digested with a restriction
10 enzyme to linearize the vector. PCR amplification primers are designed so that the
5' ends of the PCR fragments can hybridize to the DNA sequence of the vectors. If
more than one PCR fragment is amplified, then common sequences to the two
fragments are introduced by oligonucleotides. Bacteria are transfected with the PCR
fragments and with the digested vector. The fragments and vector can recombine by
15 homologous recombination using the bacteria's recombination machinery. Bacterial
colonies are selected and the DNA is analyzed by size and restriction map as a
preliminary determination that the vector and fragment(s) recombined correctly.
Correct insertion of fragments with the mutations is confirmed by dideoxynucleotide
sequence analysis. DNA is then introduced into mammalian cells as described for
20 the CH2 deleted antibody, and the expressed antibody analyzed for binding and
functional activity.

By way of example, mutations Leu to Ala at residue 235 in CH2 and Gly to Ala at
residue 237 were introduced by the procedure disclosed in Example 4. The heavy
25 chain vector used for this procedure was pD17-hG1a, similar to pD17-BR96 vector
described herein except that humanized V regions (Rosok, M.J., D.E. Yelton, L.J.
Harris, J. Bajorath, K-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin,
W.D. Huse, and S.M. Glaser, 1996. J. Biol. Chem 271 37:22611-22618) with three
affinity mutations (H1, H2, and H3 mutations) were substituted.

pBR96-hG1a contains two *Eco*47-III restriction sites flanking the Ig hinge-CH2-CH3 domains. The recipient vector was prepared by (1) digesting pBR96-hG1a with *Eco*47-III, (2) isolating the vector by agarose gel electrophoresis, and (3)
5 extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA). To introduce mutations at a single location, such as for positions 235 and 237, two PCR products were synthesized.

To introduce two distally located mutations, such as for mutant F (also referred to
10 herein as hBR96-2F) with mutations at 235, 237, 331, requires 3 PCR products. The recombination of neighboring PCR products occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends contain complementary mutant residues. The mutagenic PCR primers contain at least 15
15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers containing sequences for recombining with the end regions of the *Eco*47-III digested pBR96-hG1a vector.

PCR amplification used 250 ng intact pBR96-hG1a DNA template, 10 μ l of 10X *Pfu*
20 buffer (Stratagene, Inc., San Diego, CA), 10 nmol dNTPs, 200 ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase (Stratagen, Inc. San Diego, CA) in 100 μ l reaction. Samples were denatured at 95°C for 5 min, annealed at 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45
25 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, and a final extension at 72°C for 7 min. The amplified products were purified from a 1% agarose gel, extracted with the Qiagen Gel Extraction kit and quantitated. 50 mg of each PCR product was mixed with 25 ng of the *Eco*47-III digested pBR96-hG1a vector and transfected in E.coli MAX Efficiency DH5 α TM according to the

manufacturer's instructions (GIBCO BRL/Life Technologies, Gaithersburg, MD).

The entire transfection reaction was plated onto LB agar plated containing 100 µg/ml ampicillin.

- 5 Bacterial colonies were selected and grown overnight at 37° C in 2 ml liquid cultures. DNA was isolated and analyzed by Eco47-III restriction endonuclease mapping. Clones with the correct size insert were sequenced (Sequenase Version 2, U.S. Biochemical Corp., Cleveland, OH).
- 10 The second method for introducing site specific mutations into the CH₂ domain of human IgG1 involved the method of Kunkel (1987 Methods Enzymology, supra). For this procedure pD17-hG1b DNA with the F1 origin of replication was introduced into electrocompetent E. coli CJ236 dut-ung- (Bio-Rad Laboratories, Hercules, CA) by electroporation according to manufacturer's instructions. PD17-
- 15 hG1b is a vector having a constant region but no variable region. The F1 ori site allows treatment of this vector as a phagemid.

- Bacteria containing the plasmid were selected by ampicillin resistance. Single stranded uridinylated DNA was prepared using the Muta-Gene Phagemid In Vitro
- 20 Mutagenesis Version 2 protocol (Bio-Rad). Mutations were introduced by site-directed mutagenesis with the appropriate antisense oligonucleotide. For molecules with mutations at more than one location, mutations were introduced by either of the two methods discussed above. One method would be to (1) prepare one mutant, for example, mutant 2C (also referred to herein as BR96-2C) with the mutations at
 - 25 residues 318, 320, 322, (2) isolate ssDNA, and (3) introduce a second mutation set with the appropriate anti-sense oligonucleotide. The second method would be to anneal two antisense oligonucleotides with the same uridinylated ssDNA and screen for mutants with both sets of changes. Mutant 2H (hBR96-2H) was also prepared by a combination of these methods.

The V region of humanized BR96-2 heavy chain was introduced by the homologous recombination method described above in pD17-hJm14.H1. The pD17-hJm14.H1 plasmid contains the BR96 humanized variable region with the H1/H2/H3

5 mutations and the plasmid was used to transfect mutant sequences into mammalian cells. The pD17G1b vector containing the Fc mutation(s) was digested with NheI for 3 hr at 37° C and the DNA isolated by methods described above. Insertion of the V region into the vector was determined by size and restriction enzyme mapping and confirmed by sequence analysis.

10

Transient expression of whole antibodies was performed by transfection of COS cells. For production of antibody, stable transfections of CHO cells were performed (see description of deleted CH2 mutant). All mutants were purified from CHO culture supernatants by protein A chromatography.

15

The oligonucleotide primers homologous to the vector and used to introduce the constant regions mutations were as follows:

Oligonucleotides homologous to vector sequences:

Sens(sense)CH2 E47-3-5: CAG GGA GGG AGG GTG TCT GCT GGA AGC

20 CAG GCT CAG CGC TGA CCT CAGA

D.CH2 E47-3 A (antisense): GGA AAG AAC CAT CAC AGT CTC GCA GGG
GCC CAG GGC AGC GCT GGG TGC TT

Oligonucleotides to mutate Leu235 to Ala and Gly237 to Ala (underlined sequences

25 show sites of mutation):

Antisense CH2 L235-G237/aa: GAA GAG GAA GAC TGA CGG TGC CCC
CGC GAG TTC AGG TGC TGA GG

SensCH2 L235-G237/AA: CCT CAG CAC CTG AAC TCG CGG GGG CAC
CGT CAG TCT TCC TCT TC

Oligonucleotides to mutate Glu318, Lys320, Lys322 to Ser

Antis(antisense)CH2 EKK/SSS-2: CTG GGA GGG CTT TGT TGG AGA CCG
AGC ACG AGT ACG ACT TGC CAT TCA GCC

- 5 Oligonucleotides to mutate Pro331 to Ala:

Antis CH2 P331/A/3: GAT GGT TTT CTC GAT GGC GGC TGG GAG GGC

Sense CH2 P33/A: GCC CTC CCA GCC GCC ATC GAG AAA ACC ATC

Alternative antisense oligo to introduce Ala at 331 by site-directed mutation:

CH2P331A: GAT GGT TTT CTC GAT AGC GGC TGG GAG GGC TTT G

10

Oligonucleotides to mutate Glu318 to Ser, Lys320 to Ser, Lys322 to Ser, and Pro331 to Ala:

Antis CH2 EKKP/SSA-6: GAT GGT TTT CTC GAT GGC GGC TGG GAG
 GGC TTT GTT GGA GAC CGA GCA CGA GTA CGA CTT GCC ATT CAG

15 CCA GTC CTG GTG

Sense CH2 EKKP/SSA-6: CAC CAG GAC TGG CTG AAT GGC AAG TCG
 TAC TCG TGC TCG GTC TCC AAC AAA GCC CTC CCA GCC GCC ATC
 GAG AAA ACC ATC

20

In vitro Assays of the Mutants

Results of the CDC demonstrate that mutant hBR96-2B has approximately 10 fold less activity than the control hBR96-1 (two affinity mutations, one in H2 and one in H3, refer to previous patent (Figure 20)). The mutants that have the least ability to kill cells in the presence of complement is hBR96-2C with the triple mutations at positions 318, 320, and 322 and the hBR96-2H mutant (least cytotoxic antibodies in the panel) which contains all six mutations at the three different locations. ADCC activity was most affected by the CH2 deleted hBR96-2 molecule (Figure 21).

hBR96-2B and -2H lost between 100 and 1000 fold activity to kill in the presence of effector cells. In the ADCC assay the hBR96-2B molecule also lost approximately 10 fold activity (Figure 21).

- 5 Figures 26-28 provide the amino acid sequences for the heavy chain variable region for both chimeric and humanized BR96 having the H1, H2, and H3 mutations. The amino acid sequence for the light chain variable region is known and methods for generating it are found in PCT Application No. 95/305444. Additionally provided is the amino acid sequence for the IgG1 constant region. Mutations in the constant
- 10 region are marked.

SEQUENCE LISTING

(1) GENERAL INFORMATION

5

(i) APPLICANT: Bristol-Myers Squibb Co.

(ii) TITLE OF THE INVENTION:

10

A METHOD FOR INHIBITING
IMMUNOGLOBULIN-INDUCED TOXICITY FROM THE USE OF
IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS

(iii) NUMBER OF SEQUENCES: 13

15

(iv) CORRESPONDENCE ADDRESS:

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20

(v) COMPUTER READABLE FORM:

25

(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 2.0

30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT/US97/_____
(B) FILING DATE: 01-AUG-1997
(C) CLASSIFICATION:

35

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 60/023,033
(B) FILING DATE: 02-AUG-1996

40

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45

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50

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

55

(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5 TGGCACCAGAA AGCTTTCTGG GGCAGGCCAG GCCTGA 36

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

20 TCCGGACATG TTGGTACCCA CGTGGTGGTC GACGCTGAGC CTGGCTTCGA GCAGACA 57

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCCGACCACC ACGTGGGTAC CAACATGTCC GGAGCCACAT GGACAGAGGC CGGCT 55

35 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTGGTTCTTG TTCATCTCCT CTCTAGATGG 30

50 (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACCATGGTCG ACCTCAGACC TGCCAAGAGC CATATC

36

(2) INFORMATION FOR SEQ ID NO:6:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

15

CATGGTCACG TGGTGTGTCC CTGGATGCAG GCTACTCTA

39

(2) INFORMATION FOR SEQ ID NO:7:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 49 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

25

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

30

CAGGGAGGGA GGGTGTCTGC TGGAAGCCAG GCTCAGCGCT GACCTCAGA

49

(2) INFORMATION FOR SEQ ID NO:8:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 50 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

45

GGAAAGAACC ATCACAGTCT CGCAGGGGCC CAGGGCAGCG CTGGGTGCTT

50

(2) INFORMATION FOR SEQ ID NO:9:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8691 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCCG GCTTCGAATA GCCAGAGTAA
CCTTTTTTTT TAATTTTATT TTATTTTATT TTTGAGATGG AGTTTGCGC CGATCTCCCG

60

120

	ATCCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGCGC	GAGCAAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGCGC	300
	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAT	360
5	AGTAATCAAT	TACGGGGTCA	TTAGTTTATA	GCCCATATAT	GGAGTTCGCG	GTTACATAAC	420
	TTACGGTAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTTACGGTA	AACTGCCCCA	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCC	CCTGGCATTG	TGCCCAGTAC	ATGACCTTAT	660
10	GGGACTTTCC	TACTTGCCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	720
	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
15	TTAATACGAC	TCACTATAGG	GAGACCCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCCT	TGTTTTAAAA	GGTGTCCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCACTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATTA	CATGTATTGG	GTTTCGCCAGA	1260
20	TCCAGAGAA	GAGGCTGGAG	TGGGTCGCAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	1320
	ATCCAGACAC	TGTAAAGGGT	CGATTACCA	TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	1380
	ACCTGCAAAT	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
	CTAGCACCAA	GGGCCCATCG	GTCTTCCCCC	TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
25	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCCTGT	ACGGTGTCTG	1620
	GGAACCTCAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCTTA	CAGTCTCAG	1680
	GACTCTACTC	CCTCAGCAGC	GTGGTCAACG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
	ACATCTGCAA	CGTGAATCAC	AAGCCAGCA	ACACCAAGGT	GGACAAGAAA	GTTGGTGAGA	1800
	GGCCAGCACA	GGGAGGGAGG	GTGCTGTCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGACG	1860
30	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCCGTCTG	CCTCTTACCC	1920
	CGGAGGGCTC	TGCCCGCCCC	ACTCATGTCT	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG	1980
	GCTCTGGGCA	GGCAGAGGCT	AGGTGCCCCC	ACTCCAGGCC	CTGCACACAA	AGGGTGACGT	2040
	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCCCTGA	CCTAAGCCCA	2100
	CCCCAAAGGC	CAAACCTCTC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
35	GTAACCTCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTG	ACAAAACCTCA	CACATGCCCA	2220
	CCGTGCCCAG	GTAAGCCAGC	CCAGGCCTCG	CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	2280
	AGAGTAGCCT	GCATCCAGGG	ACAGGCCCCA	GCCGGGTGCT	GACACGTCCA	CCTCCATCTC	2340
	TTCTCTGACCA	CCTGAACCTC	TGGGGGGACC	CTCAGTCTTC	CTCTTCCCCC	CAAAACCCAA	2400
	GGACACCCTC	ATGATCTCCC	GGACCCCTGA	GGTCACATGC	GTGGTGGTGG	ACGTGAGCCA	2460
40	CGAAGACCCT	GAGGTCAAGT	TCAACTGGTA	CGTGGACGGC	GTGGAGGTGC	ATAATGCCAA	2520
	GACAAAGCCG	CGGGAGGAGC	AGTACAACAG	CACGTACCGT	GTGGTCAGCG	TCCTCACCGT	2580
	CCTGCACCAG	GACTGGCTGA	ATGGCAAGGA	GTACAAGTGC	AAGGTCTCCA	ACAAAGCCCT	2640
	CCCAGCCCCC	ATCGAGAAAA	CCATCTCCAA	AGCCAAAGGT	GGGACCCGTG	GGGTGCGAGG	2700
	GCCACATGGA	CAGAGGCCGG	CTCGGCCAC	CCTTGCCCT	GAGAGTGACC	GCTGTACCAA	2760
45	CCTCTGTCCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCCTGCCC	CCATCCCGGG	2820
	ATGAGCTGAC	CAAGAACCAG	GTGAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCAGCG	2880
	ACATCGCCGT	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCTC	2940
	CCGTGCTGGA	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GCTCACCGTG	GACAAGAGCA	3000
	GGTGGCAGCA	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	3060
50	ACACGCAGAA	GAGCCTCTCC	CTGTCTCCGG	GTAATGAGT	GCGACGGCCG	GCAAGCCCCC	3120
	GCTCCCCGGG	CTCTCGCGGT	CGCACGAGGA	TGCTTGGCAC	GTACCCCTGT	TACATACTTC	3180
	CCGGGCGCCC	AGCATGGAAA	TAAAGCACCC	AGCGCTGCC	TGGGCCCTGT	CGAGACTGTG	3240
	ATGGTTCTTT	CCACGGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	3300
	GGGTCCCACT	GTCCCCACAC	TGGCCAGGCG	TGTGCAGGTG	TGCCTGGGCC	CCCTAGGGTG	3360
55	GGGCTCAGCC	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	3420
	AGCAGCACCT	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCTGGGG	GACAGACACA	3480
	CAGCCCCCTG	CTGTGTAGGA	GACTGTCTGT	TTCTGTGAGC	GCCCCGTGTC	TCCCGACCTC	3540
	CATGCCCACT	CGGGGCGATG	CCTAGTCCAT	GTGCGTAGGG	ACAGGCCCTC	CCTACCCCAT	3600
	CTACCCCCAC	GGCACTAACC	CCTGGCTGCC	CTGCCAGGCC	TCGCACCCGC	ATGGGGACAC	3660

	AACCGACTCC	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GACTGGTGCA	GATGCCCCACA	3720
	CACACACTCA	GCCCCAGACCC	GTTCAACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	3780
	CACCACACAC	ACACGTGCAC	GCCTCACACA	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	3840
	CCCAGACCAG	AGCAAGGTCC	TCGCACACGT	GAACACTCCT	CGGACACAGG	CCCCACGAG	3900
5	CCCCACGCGG	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	3960
	TCAGACAAAC	CCAGCCCTCC	TCTCACAAGG	GTGCCCCTGC	AGCCGCCACA	CACACACAGG	4020
	GGATCACACA	CCACGTCACG	TCCCTGGCCC	TGGCCCACTT	CCCAGTGCCG	CCCTTCCCTG	4080
	CAGGACGGAT	CAGCCTCGAC	TGTGCCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCTCC	4140
	CCCGTGCCTT	CCTTGACCCT	GGAAGGTGCC	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	4200
10	GAAATTGCAT	CGCATTGTCT	GAGTAGGTGT	CATTCTATTC	TGGGGGGTGG	GGTGGGGCAG	4260
	GACAGCAAGG	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	4320
	ATGGCTTCTG	AGGCGGAAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGCGCCCTGT	4380
	AGCGGCGCAT	TAAGCGCGGC	GGGTGTGGTG	GTTACGCGCA	GCGTGACCGC	TACACTTGCC	4440
	AGCGCCCTAG	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCCT	TTCTCGCCAC	GTTCGCCGGG	4500
15	CCTCTCAAAA	AAGGGAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	4560
	CTAACTCCGC	CCATCCCGCC	CCTAACTCCG	CCCAGTTCCG	CCCATTCTCC	GCCCCATGGC	4620
	TGACTAATTT	TTTTTATTTA	TGCAGAGGCC	GAGGCCGCCT	CGGCCTCTGA	GCTATTCCAG	4680
	AAGTAGTGAG	GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	4740
	GCTCGGATTT	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	4800
20	CCCGCTGCCA	TCATGGTTTCG	ACCATTGAAC	TGCATCGTCG	CCGTGTCCCA	AAATATGGGG	4860
	ATTGGCAAGA	ACGGAGACCT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	4920
	AGAATGACCA	CAACCTCTTC	AGTGGAAGGT	AAACAGAATC	TGGTGATTAT	GGGTAGGAAA	4980
	ACCTGGTTCT	CCATTCTCTGA	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	5040
	AGTAGAGAAC	TCAAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTTGATGAT	5100
25	GCCTTAAGAC	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	5160
	GGAGGCGAGT	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	5220
	ACAAGGATCA	TGCAGGAATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	5280
	TATAAACTTC	TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	5340
	AAGTATAAGT	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	5400
30	GCTCCCCCTC	TAAAGCTATG	CATTTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	5460
	TCTTTGTGAA	GGAACCTTAC	TTCTGTGGTG	TGACATAATT	GGACAACTA	CCTACAGAGA	5520
	TTTAAAGCTC	TAAGGTAAAT	ATAAAATTTT	TAGTGTATA	ATGTGTTAAA	CTACTGATTC	5580
	TAATTGTTTG	TGATTTTTAG	ATTTCAACCT	ATGGAAGTGA	TGAATGGGAG	CAGTGGTGGA	5640
	ATGCCTTTAA	TGAGGAAAAC	CTGTTTTGCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	5700
35	CTACTGCTGA	CTCTCAACAT	TCTACTCCTC	CAAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	5760
	AGGACTTTCC	TTCAGAATTG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTAGT	AATAGAATCT	5820
	TTGCTTGCTT	TGCTATTTAC	ACCACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAAATTA	5880
	TGGAAAAATA	TTCTGTAACC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	5940
	TTTTTCTTAC	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACATGCT	CAAAAATTGT	6000
40	GTACCTTTAG	CTTTTAAATT	TGTAAAGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCCT	6060
	TGACTAGAGA	TCATAATCAG	CCATAACCACA	TTTGTAGAGG	TTTACTTGC	TTTAAAAAAC	6120
	CTCCACACAC	TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	6180
	TTTATTGCAG	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	6240
	GCATTTTTTT	CACTGCATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	6300
45	GTCTGGATCG	GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCAC	6360
	CCCAACTTGT	TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTT	6420
	ACAAATAAAG	CATTTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	6480
	TCTTATCATG	TCTGTATACC	GTGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	6540
	CTGTTTCCCTG	TGTGAAATTG	TTATCCGCTC	ACAAATCCAC	ACAACATACG	AGCCGGAAGC	6600
50	ATAAAGTGTA	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	6660
	TCACTGCCCG	CTTTCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	6720
	CGCGCGGGGA	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG	CTTCCTCGCT	CACTGACTCG	6780
	CTGCGCTCGG	TCGTTCCGGT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	6840
	TTATCCACAG	AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAGG	CCAGCAAAAG	6900
55	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	6960
	GAGCATCACA	AAAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	7020
	TACCAAGGCGT	TTCCCCCTGG	AAGTCCCTCT	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	7080
	ACCGGATACC	TGTCGCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	7140
	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	7200

	CCCGTTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	7260
	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	7320
	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	7380
	GTATTTGGTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	7440
5	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	7500
	ACGCGCAGAA	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	7560
	CAGTGGAACG	AAAACCTCAC	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	7620
	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	7680
	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	7740
10	TTTCGTTTCAT	CCATAGTTGC	CTGACTCCCC	GTCTGTGATA	TAACTACGAT	ACGGGAGGGC	7800
	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	7860
	TTATCAGCAA	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	7920
	TCCGCCTCCA	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCGCCAGTT	7980
	AATAGTTTGC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACAG	CTCGTCGTTT	8040
15	GGTATGGCTT	CATTCACTGC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	8100
	TTGTGCAAAA	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTGAGAAG	TAAGTTGGCC	8160
	GCAGTGTTAT	CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	8220
	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	8280
	CGGCGACCGA	GTTGCTCTTG	CCC GGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	8340
20	ACTTTAAAAG	TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GAAAACTCTC	AAGGATCTTA	8400
	CCGCTGTTGA	GATCCAGTTC	GATGTAACCC	ACTCGTGCAC	CCAACTGATC	TTCAGCATCT	8460
	TTTACTTTCA	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAG	8520
	GGAATAAGGG	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTTC	ATATTATTGA	8580
	AGCATTTATC	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	8640
25	AAACAAATAG	GGGTTCCGCG	CACATTTCCC	CGAAAAGTGC	CACCTGACGT	C	8691

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8327 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

	GACGGATCGG	GAGATCTGCT	AGGTGACCTG	AGGCGCGCCG	GCTTCGAATA	GCCAGAGTAA	60
40	CCTTTTTTTT	TAATTTTATT	TTATTTTATT	TTTGAGATGG	AGTTTGCGCG	CGATCTCCCG	120
	ATCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGCGC	GAGCAAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGCGC	300
	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAT	360
45	AGTAATCAAT	TACGGGGTCA	TTAGTTCATA	GCCCATATAT	GGAGTTCCGC	GTTACATAAC	420
	TTACGGTAA	TGCCCCGCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTACGGTA	AACTGCCCC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCC	CCTGGCATT	TGCCCAGTAC	ATGACCTTAT	660
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	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
55	TTAATACGAC	TCACTATAGG	GAGACCCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTTGTTTAA	GCTTGGTCTT	TCCTTGCTCT	TGTTTTAAAA	GGTGTCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCCTGTGT	AACCTCTGGA	TTCACCTTCA	GTGACTATTA	CATGTATTGG	GTTCCGCAGA	1260

	CTCCAGAGAA	GAGGCTGGAG	TGGGTCGCAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	1320
	ATCCAGACAC	TGTAAAGGGT	CGATTACCA	TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	1380
	ACCTGCAAAT	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
5	CTAGCACCAA	GGGCCCATCG	GTCTTCCCCC	TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCGGTG	ACGGTGTCTG	1620
	GGAACTCAGG	CGCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCCCTA	CAGTCCCTCAG	1680
	GACTCTACTC	CCTCAGCAGC	GTGGTCAACG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
	ACATCTGCAA	CGTGAATCAC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTTGGTGAGA	1800
10	GGCCAGCACA	GGGAGGGAGG	GTGTCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGACG	1860
	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCCGTCTG	CCTCTTCACC	1920
	CGGAGGCGCT	TGCCCCGCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG	1980
	GCTCTGGGCA	GGCAGAGGCT	AGGTGCCCCT	AACCCAGGCC	CTGCACACAA	AGGGGCAGGT	2040
	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCTGA	CCTAAGCCCA	2100
15	CCCCAAGGC	CAAACTCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
	GTAACCTCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTC	ACAAAACCTCA	CACATGCCCA	2220
	CCGTGCCCCAG	GTAAGCCAGC	CCAGGCCTCG	CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	2280
	AGAGTAGCCT	GCATCCAGGG	ACACACCACG	TGGGTACCAA	CATGTCCGGA	GCCACATGGA	2340
	CAGAGGCGCG	CTCGGCCCC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	CCTCTGTCCC	2400
20	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCTTGCCC	CCATCCCGGG	ATGAGCTGAC	2460
	CAAGAACCAG	GTCAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCCAGCG	ACATCGCCGT	2520
	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCTC	CCGTGCTGGA	2580
	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GCTCACCCTG	GACAAGAGCA	GGTGGCAGCA	2640
	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	ACACGCAGAA	2700
25	GAGCCTCTCC	CTGTCTCCGG	GTAAATGAGT	GCGACGGCCG	GCAAGCCCCC	GCTCCCCGGG	2760
	CTCTCGCGGT	CGCACGAGGA	TGCTTGCGAC	GTACCCCTGT	TACATACTTC	CCGGGCGCCC	2820
	AGCATGGAAG	TAAAGCACCC	AGCGCTGCC	TGGGCCCCCTG	CGAGACTGTG	ATGGTTCTTT	2880
	CCACGGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	GGGTCCCACT	2940
	GTCCCCACAC	TGGCCAGGC	TGTGCAGGTG	TGCCCTGGGC	CCCTAGGGTG	GGGCTCAGCC	3000
30	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	AGCAGCACCT	3060
	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCTTGGG	GACAGACACA	CAGCCCCTGC	3120
	CTCTGTAGGA	GACTGTCTGT	TTCTGTGAGC	GCCCCGTGCC	TCCCGACCTC	CATGGCCACT	3180
	CGGGGGCATG	CCTAGTCCAT	GTGCGTAGGG	ACAGGCCCTC	CCTCACCCAT	CTACCCCCAC	3240
	GGCACTAACC	CCTGGCTGCC	CTGCCAGCC	TCGCACCCGC	ATGGGGACAC	AACCGACTCC	3300
35	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GACTGGTGCA	GATGCCCACA	CACACACTCA	3360
	GCCCAGACCC	GTTCAACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	CACCACACAC	3420
	ACACGTGCAC	GCCTCACACA	CGGAGCCTCA	CCGGGGCGAA	CTGCACAGCA	CCCAGACGAG	3480
	AGCAAGGTCC	TCGCACACGT	GAACACTCCT	CGGCACAGG	CCCCACGAG	CCCCACGGG	3540
	CACCTCAAGG	CCCCAGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	TCAGACAAAC	3600
40	CCAGCCCTCC	TCTCACAAAG	GTGCCCTGTC	AGCCGCCACA	CACACACAGG	GGATCACACA	3660
	CCACGTACAG	TCCCTGGCCC	TGGCCCACTT	CCCAGTGCCG	CCCTTCCCTG	CAGGACGGAT	3720
	CAGCCTCGAC	TGTGCCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCTCC	CCCGTGCCCT	3780
	CCTTGACCCT	GGAAGGTGCC	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	GAAATTGCAT	3840
	CGCATTGTCT	GAGTAGGTGT	CATTCTATTC	TGGGGGGTGG	GGTGGGGCAG	GACAGCAAGG	3900
45	GGGAGGATTG	GGAAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	ATGGCTTCTG	3960
	AGGCGGAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGCGCCCTGT	AGCGGCGCAT	4020
	TAAGCGCGGC	GGGTGTGGTG	GTTACGCGCA	GCGTGACCGC	TACACTTGCC	AGCGCCCTAG	4080
	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCCT	TTCTCGCCAC	GTTGCGCCGG	CCTCTCAAAA	4140
	AAGGGAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	CTAACTCCGC	4200
50	CCATCCCGCC	CCTAACTCCG	CCCAGTTCGG	CCCATTCTCC	GCCCCATGGC	TGACTAATTT	4260
	TTTTTATTTA	TGCAGAGGCC	GAGGCCGCCCT	CGGCCTCTGA	GCTATTCCAG	AAGTAGTGAG	4320
	GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	GCTGCGATTT	4380
	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	CCCGCTGCCA	4440
	TCATGTTTCG	ACCATTGAAC	TGCATCGTCG	CCGTGTCCCA	AAATATGGGG	ATTGGCAAGA	4500
55	ACGGAGACCT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	AGAATGACCA	4560
	CAACCTCTTC	AGTGGAAAGT	AAACAGAATC	TGGTGATTAT	GGGTAGGAAA	ACCTGGTTCT	4620
	CCATTCTCTG	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	AGTAGAGAAC	4680
	TCAAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTTGGATGAT	GCCTTAAGAC	4740
	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	GGAGGCAGTT	4800

	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	ACAAGGATCA	4860
	TGCAGGAATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	TATAAACTTC	4920
	TCCCAGAAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	AAGTATAAGT	4980
	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	GCTCCCCCTCC	5040
5	TAAAGCTATG	CATTTTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTTAGATC	TCTTTGTGAA	5100
	GGAACCTTAC	TTCTGTGGTG	TGACATAATT	GGACAACTA	CCTACAGAGA	TTTAAAGCTC	5160
	TAAGGTAAAT	ATAAAATTTT	TAAGTGATATA	ATGTGTTAAA	CTACTGATTTC	TAATTGTTTG	5220
	TGTATTTTAG	ATTCCAACCT	ATGGAAGTGA	TGAATGGGAG	CAGTGGTGGA	ATGCCTTTTAA	5280
	TGAGGAAAAC	CTGTTTTGCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	CTACTGCTGA	5340
10	CTCTCAACAT	TCTACTCCTC	CAAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	AGGACTTTTCC	5400
	TTCAGAAATTG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTTAGT	AATAGAACTC	TTGCTTGCTT	5460
	TGCTATTTAC	ACCACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAATTA	TGGAATAATA	5520
	TTCTGTAAAC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	TTTTTCTTAC	5580
	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	CAAAAATTGT	GTACCTTTAG	5640
15	CTTTTTAATT	TGTAAAGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCCCT	TGACTAGAGA	5700
	TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTACTTGC	TTTAAAAAAC	CTCCACACC	5760
	TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	TTTATTGCAG	5820
	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	GCATTTTTTT	5880
	CACGTGCATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	GTCTGGATCG	5940
20	GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCAC	CCCAACTTGT	6000
	TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTT	ACAAATAAAG	6060
	CATTTTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	TCTTATCATG	6120
	TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCCTG	6180
	TGTGAAATTG	TTATCCGCTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	ATAAAGTGTG	6240
25	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	TCAGTGCCCG	6300
	CTTTCCAGTG	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA	6360
	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG	CTTCTCGCT	CACTGACTCG	CTGCGCTCGG	6420
	TCGTTCCGGCT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	6480
	AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	6540
30	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCCTGAC	GAGCATCACA	6600
	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	6660
	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	6720
	TGTCGCGCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	TGTAGGTATC	6780
	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	6840
35	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	6900
	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGGT	6960
	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTTGGTA	7020
	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	7080
	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	7140
40	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAAACG	7200
	AAAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC	7260
	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG	7320
	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTAT	7380
	CCATAGTTGC	CTGACTCCCC	GTGCTGTAGA	TAACTACGAT	ACGGGAGGGC	TTACCATCTG	7440
45	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCAC	GGCTCCAGAT	TTATCAGCAA	7500
	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	TCCGCTCCA	7560
	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCGCCAGTT	AATAGTTTGC	7620
	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACG	CTCGTCGTTT	GGTATGGCTT	7680
	CATTACAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGTGCAAAA	7740
50	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAGTTGGCC	GCAGTGTTAT	7800
	CACCTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	GTAAGATGCT	7860
	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGCGACCGA	7920
	GTTGCTCTTG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAAG	7980
	TGCTCATCAT	TGGAAACGT	TCTTCGGGGC	GAAAACTCTC	AAGGATCTTA	CCGCTGTGTA	8040
55	GATCCAGTTC	GATGTAACCC	ACTCGTGCAC	CCAACGTATC	TTCAGCATCT	TTTACTTTCA	8100
	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAAG	GGAATAAGGG	8160
	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCTTTTTCAT	ATATTATGTA	AGCATTTATC	8220
	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG	8280
	GGGTTCCGCG	CACATTTCCC	CGAAAAGTGC	CACCTGACGT	CCBRAAG		8327

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 8897 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

	GGTACCAATT	TAAATTGATA	TCTCCTTAGG	TCTCGAGCAC	CATGAAGTTG	CCTGTTAGGC	60
15	TGTTGGTGCT	GATGTTCTGG	ATTCCTGCTT	CCAGCAGTGA	TGTTTTGATG	ACCCAAATTC	120
	CAGTCTCCCT	GCCTGTCAGT	CTTGGAGATC	AAGCGTCCAT	CTCTTGCAGA	TCTAGTCAGA	180
	TCATTGTACA	TAATAATGGC	AACACCTATT	TAGAATGGTA	CCTGCAGAAA	CCAGGCCAGT	240
	CTCCACAGCT	CCTGATCTAC	AAAGTTTCCA	ACCGATTTTC	TGGGGTCCCA	GACAGGTTCA	300
	GCGGCAGTGG	ATCAGGGACA	GATTTACAC	TCAAGATCAG	CAGAGTGGAG	GCTGAGGATC	360
20	TGGGAGTTTA	TTACTGCTTT	CAAGGTTTAC	ATGTTCCATT	CACGTTCCGC	TCGGGGACAA	420
	AGTTGGAAAT	AAAACGTAAG	TCTCGAGTCT	CTAGATAACC	GGTCAATCGA	TTGGAATTCT	480
	AAACTCTGAG	GGGGTCGGAT	GACGTGGCCA	TTCTTTGCC	AAAGCATTGA	GTTTACTGCA	540
	AGGTCAGAAA	AGCATGCAAA	GCCCTCAGAA	TGGCTGCAAA	GAGCTCCAAC	AAAACAATTT	600
	AGAACCTTAT	TAAGGAATAG	GGGGAAGCTA	GGAAGAAACT	CAAAACATCA	AGATTTTAAA	660
25	TACGCTTCTT	GGTCTCCTTG	CTATAATTAT	CTGGGATAAG	CATGCTGTTT	TCTGTCTGTC	720
	CCTAACATGC	CCTTATCCGC	AAACAACACA	CCCAAGGGCA	GAACCTTGTT	ACTTAAACAC	780
	CATCCTGTTT	GCTTCTTTCC	TCAGGAACTG	TGGCTGCACC	ATCTGTCTTC	ATCTTCCCGC	840
	CATCTGATGA	GCAGTTGAAA	TCTGGAAGT	CCTCTGTTGT	GTGCCTGCTG	AATAACTTCT	900
	ATCCCAGAGA	GGCCAAAGTA	CAGTGGGAAG	TGGATAACGC	CCTCCAATCG	GGTAACTCCC	960
30	AGGAGAGTGT	CACAGAGCAG	GAGAGCAAGG	ACAGCACCTA	CAGCCTCAGC	AGCACCCCTGA	1020
	CGCTGAGCAA	AGCAGACTAC	GAGAAACACA	AAGTCTACGC	CTGCGAAGTC	ACCCATCAGG	1080
	GCCTGAGCTC	GCCCGTCACA	AAGAGCTTCA	ACAGGGGAGA	GTGTTAGAGG	GAGAAGTGCC	1140
	CCCACCTGCT	CCTCAGTTCC	AGCCTGACCC	CCTCCCATCC	TTTGGCCTCT	GACCCTTTTT	1200
	CCACAGGGGA	CCTACCCCTA	TTGCGGTCCT	CCAGCTCATC	TTTCACTCA	CCCCCTCCT	1260
35	CCTCCTTGGC	TTTAATTATG	CTAATGTTGG	AGGAGAATGA	ATAAATAAAG	TGAATCTTTG	1320
	CACCTGTGGT	TTCTCTCTTT	CCTCATTTAA	TAATTATTAT	CTGTTGTTTT	ACCAACTACT	1380
	CAATTTCTCT	TATAAGGGAC	TAAATATGTA	GTCATCCTAA	GGCACGTAAC	CATTTATAAA	1440
	AATCATCCTT	CATTCTATTT	TACCCTATCA	TCCTCTGCAA	GACAGTCCTC	CCTCAAACCC	1500
	ACAAGCCTTC	TGTCCTCACA	GTCCCTGGG	CCATGGTAGG	AGAGACTTGC	TTCTTTGTTT	1560
40	TCCCTCCTC	AGCAAGCCCT	CATAGTCCTT	TTTAAGGGTG	ACAGGTCTTA	CAGTCATATA	1620
	TCCTTTGATT	CAATTCCCTG	AGAATCAACC	AAAGCAAATT	TTTCAAAAGA	AGAAACCTGC	1680
	TATAAAGAGA	ATCATTCATT	GCAACATGAT	ATAAAATAAC	AACACAATAA	AAGCAATTAA	1740
	ATAAACAAAC	AATAGGGAAA	TGTTTAAGTT	CATCATGGTA	CTTAGACTTA	ATGGAATGTC	1800
	ATGCCTTATT	TACATTTTAA	AACAGGTACT	GAGGGACTCC	TGTCTGCCAA	GGGCCGTATT	1860
45	GAGTACTTTC	CACAACCTAA	TTTAATCCAC	ACTATACTGT	GAGATTAAAA	ACATTCATTA	1920
	AAATGTTGCA	AAGGTTCTAT	AAAGCTGAGA	GACAAATATA	TTCTATAACT	CAGCAATCCC	1980
	ACTTCTAGAT	GACTGAGTGT	CCCCACCCAC	CAAAAACTA	TGCAAGAATG	TTCAAAGCAG	2040
	CTTTATTTAC	AAAAGCCAAA	AATTGAAAAT	AGCCCGATTG	TCCAACAATA	GAATGAGTTA	2100
	TTAAACTGTG	GTATGTTTAT	ACATTAGAAT	ACCCAATGAG	GAGAATTAAC	AAGCTACAAC	2160
50	TATACCTACT	CACACAGATG	AATCTCATAA	AAATAATGTT	ACATAAGAGA	AACTCAATGC	2220
	AAAAGATATG	TTCTGTATGT	TTTCATCCAT	ATAAAGTTCA	AAACCAGGTA	AAAATAAAGT	2280
	TAGAAATTTG	GATGGAAATT	ACTCTTAGCT	GGGGGTGGGC	GAGTTAGTGC	CTGGGAGAAG	2340
	ACAAGAAGGG	GCTTCTGGGG	TCTTGGAAT	GTTCTGTTCC	TCGTGTGGGG	TTGTGCAGTT	2400
	ATGATCTGTG	CACGTGTTCTG	TATACACATT	ATGCTTCAAA	ATAACTTCAC	ATAAAGAACA	2460
55	TCTTATACCC	AGTTAATAGA	TAGAAGAGGA	ATAAGTAATA	GGTCAAGACC	AACGCAGCTG	2520
	GTAAGTGGGG	GCCTGGGATC	AAATAGCTAC	CTGCCTAATC	CTGCCWCCTT	GAGCCCTGAA	2580
	TGAGTCTGCC	TTCCAGGGCT	CAAGGTGCTC	AACAAAACAA	CAGGCCTGCT	ATTTTCCTGG	2640
	CATCTGTGCC	CTGTTTGGCT	AGCTAGGAGC	ACACATACAT	AGAAATTAAA	TGAAACAGAC	2700
	CTTCAGCAAG	GGGACAGAGG	ACAGAATTAA	CCTTGCCCAG	ACACTGGAAA	CCCATGTATG	2760

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	ACCTCTCTCT	GCCTACACTC	TGAAGGGGTT	CAGGAGTAAC	TAACACAGCA	TCCCTTCCCT	2940
5	CAAATGACTG	ACAATCCCTT	TGTCCTGCTT	TGTTTTTCTT	TCCAGTCAGT	ACTGGGAAAAG	3000
	TGGGGAAGGA	CAGTCATGGA	GAAACTACAT	AAGGAAGCAC	CTTGCCCTTC	TGCCTCTTGA	3060
	GAATGTTGAT	GAGTATCAAA	TCTTTCAAAC	TTTGGAGGTT	TGAGTAGGGG	TGAGACTCAG	3120
	TAATGTCCCT	TCCAATGACA	TGAACCTGCT	CACATCATCC	TGGGGGCCAA	ATTGAACAAT	3180
	CAAAGGCAGG	CATAATCCAG	TTATGAATTC	TTGCGGCCGC	TTGCTAGCTT	CACGTGTTGG	3240
	ATCCAACCGC	GGAAGGGCCC	TATTCTATAG	TGTCACCTAA	ATGCTAGAGC	TCGCTGATCA	3300
10	GCCTCGACTG	TGCCTTCTAG	TTGCCAGCCA	TCTGTTGTTT	GCCCCCTCCC	CGTGCCTTCC	3360
	TTGACCCTGG	AAGGTGCCAC	TCCCCTGCTC	CTTTCCTAAT	AAAATGAGGA	AATTGCATCG	3420
	CATTGTCTGA	GTAGGTGTCA	TTCTATTCTG	GGGGGTGGGG	TGGGGCAGGA	CAGCAAGGGG	3480
	GAGGATTGGG	AAGACAATAG	CAGGCATGCT	GGGGATGCGG	TGGGCTCTAT	GGCTTCTGAG	3540
	GCGGAAAGAA	CCAGCTGGGG	CTCTAGGGGG	TATCCCCACG	CGCCCTGTAG	CGGCGCATTA	3600
15	AGCGCGGCGG	GTGTGGTGGT	TACGCGCAGC	GTGACCGCTA	CACTTGCCAG	CGCCCTAGCG	3660
	CCCCTCCTT	TCGCTTTCTT	CCCTTCCTTT	CTCGCCACGT	TCGCCGGGCC	TCTCAAAAAA	3720
	GGGAAAAAAA	GCATGCATCT	CAATTAGTCA	GCAACCATAG	TCCC GCCCCT	AACTCCGCC	3780
	ATCCCCCCCC	TAACCTCCGC	CAGTTCCGCC	CATTCTCCGC	CCCATGGCTG	ACTAATTTTT	3840
	TTTATTTATG	CAGAGGCCGA	GGCCGCCCTG	GCCTCTGAGC	TATTCCAGAA	GTAGTGAGGA	3900
20	GGCTTTTTTG	GAGGCCCTAGG	CTTTTGCAAA	AAGCTTGGAC	AGCTCAGGGC	TGCGATTTTCG	3960
	CGCCAAACTT	GACGGCAATC	CTAGCGTGAA	GGCTGGTAGG	ATTTTATCCC	CGCTGCCATC	4020
	ATGGTTTCGAC	CATTGAACTG	CATCGTCGCC	GTGTCCCAA	ATATGGGGAT	TGGCAAGAAC	4080
	GGAGACCTAC	CCTGGCCTCC	GCTCAGGAAC	GAGTTCAAAGT	ACTTCCAAAG	AATGACCACA	4140
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25	ATTCCTGAGA	AGAATCGACC	TTTAAAGGAC	AGAATTAATA	TAGTTCTCAG	TAGAGAACTC	4260
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	ATTGAACAAC	CGGAATTGGC	AAGTAAAGTA	GACATGGTTT	GGATAGTCGG	AGGCAGTTCT	4380
	GTTTACCAGG	AAGCCATGAA	TCAACCAGGC	CACCTTAGAC	TCTTTGTGAC	AAGGATCATG	4440
	CAGGAATTTG	AAAGTGACAC	GTTTTTCCCA	GAAATTGATT	TGGGGAAAATA	TAAACTTCTC	4500
30	CCAGAATACC	CAGGCGTCCT	CTCTGAGGTC	CAGGAGGAAA	AAGGCATCAA	GTATAAGTTT	4560
	GAAGTCTACG	AGAAGAAAGA	CTAACAGGAA	GATGCTTTCA	AGTTCTCTGC	TCCCCTCCTA	4620
	AAGCTATGCA	TTTTTATAAG	ACCATGGGAC	TTTTGCTGGC	TTTAGATCTC	TTTGTGAAGG	4680
	AACCTTACTT	CTGTGGTGTG	CAATAATTGG	AAAACTACC	TACAGAGATT	TAAAGCTCTA	4740
	AGGTAAATAT	AAAATTTTTA	AGTGTATAAT	GTGTTAAACT	ACTGATTCTA	ATTGTTTGTG	4800
35	TATTTTAGAT	TCCAACCTAT	GGAAGTATG	AATGGGAGCA	GTGGTGGAAT	GCCTTTAATG	4860
	AGGAAAACCT	GTTTTTGCTCA	GAAGAAATGC	CATCTAGTGA	TGATGAGGCT	ACTGCTGACT	4920
	CTCAACATTC	TACTCCTCCA	AAAAAGAAGA	GAAAGGTAGA	AGACCCCAAG	GACTTTCCTT	4980
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40	CTGTAACCTT	TATAAGTAGG	CATAACAGTT	ATAATCATAA	CATACTGTTT	TTTCTTACTC	5160
	CACACAGGCA	TAGAGTGTCT	GCTATTAATA	ACTATGCTCA	AAAATTGTGT	ACCTTTAGCT	5220
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	ATAATCAGCC	ATACCACATT	TGTAGAGGTT	TACTTGTCTT	TAAAAAACCT	CCCACACCTC	5340
	CCCCTGAACC	TGAAACATAA	AATGAATGCA	ATTGTTGTTG	TTAATTGTTT	TATTGCAGCT	5400
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	TTCCAGTCGG	GAAACCTGTC	GTGCCAGCTG	CATTAATGAA	TCGGCCAACG	CGCGGGGAGA	5940
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55	GTTCCGGCTGC	GGCGAGCGGT	ATCAGCTCAC	TCAAAGGCGG	TAATACGGTT	ATCCACAGAA	6060
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	AATCGACGCT	CAAGTCAGAG	GTGCGGAAAC	CCGACAGGAC	TATAAGATA	CCAGGCGTTT	6240
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 TCTCTGGCTA ACTAGAGAAC CCACTGCTTA CTGGCTTATC GAAATTAATA CGACTCACTA 8880
 45 TAGGGAGACC CAAGCTT 8897

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

50

- (A) LENGTH: 8321 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC GGTCAATCGA 60
 TTGGAATTCT TGCGGCCGCT TGCTAGCCAC CATGGAGTTG TGTTTAAGCT TGGTCTTCCT 120

	TGCTCTTGTT	TTAAAAGGTG	TCCAGTGTGA	AGTGCAACTG	GTGGAGTCTG	GGGGAGGCTT	180
	AGTGCAGCCT	GGAGGGTCCC	TGCGACTTTC	CTGTGCTGCA	TCTGGATTCC	CGTTCAGTGA	240
	CTATTACATG	TATTGGGTTC	GCCAGGCTCC	AGGCAAGGGA	CTGGAGTGGG	TCTCATACAT	300
	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT	TCACCATCTC	360
5	CAGAGACAAT	GCAAAGAACA	GCCTGTACCT	GCAAATGAAC	AGCCTGAGGG	ACGAGGACAC	420
	AGCCGTGTAT	TACTGTGCAA	GAGGCCTGGC	GGACGGGGCC	TGGTTTGCTT	ACTGGGGCCA	480
	AGGGACTCTG	GTCACGGTCT	CTTCCGCTAG	CACCAAGGGC	CCATCGGTCT	TCCCCCTGGC	540
	ACCCTCCTCC	AAGAGCACCT	CTGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACTA	600
	CTTCCCCGAA	CCGGTGACGG	TGTCGTGGAA	CTCAGGCGCC	CTGACCAGCG	GCGTGCACAC	660
10	CTTCCCGGCT	GTCCTACAGT	CCTCAGGACT	CTACTCCCTC	AGCAGCGTGG	TCACCGTGCC	720
	CTCCAGCAGC	TTGGGCACCC	AGACCTACAT	CTGCAACGTG	AATCACAAGC	CCAGCAACAC	780
	CAAGGTGGAC	AAGAAAGTTG	GTGAGAGGCC	AGCAGAGGGA	GGGAGGGTGT	CTGCTGGAAG	840
	CCAGGCTCAG	CGCTCCTGCC	TGGACGCATC	CCGGCTATGC	AGCCCCAGTC	CAGGGCAGCA	900
	AGGCAGGCCC	CGTCTGCCTC	TTCACCCGGA	GGCCTCTGCC	CGCCCCACTC	ATGCTCAGGG	960
15	AGAGGGTCTT	CTGGCTTTTT	CCCCAGGCTC	TGGGCAGGCA	CAGGCTAGGT	GCCCCCTAAC	1020
	CAGGCCCTGC	ACACAAAGGG	GCAGGTGCTG	GGCTCAGACC	TGCCAAGAGC	CATATCCGGG	1080
	AGGACCCTGC	CCCTGACCTA	AGCCACCCCC	AAAGGCCAAA	CTCTCCACTC	CCTCAGCTCG	1140
	GACACCTTCT	CTCCTCCCAG	ATTCCAGTAA	CTCCCAATCT	TCTCTCTGCA	GAGCCCCAAT	1200
	CTTG TGACAA	AACTCACACA	TGCCCCACGT	GCCCAGGTAA	GCCAGCCCAG	GCCTCGCCCT	1260
20	CCAGCTCAAG	GCGGGACAGG	TGCCCTAGAG	TACCCTGCAT	CCAGGGACAC	ACCACGTGGG	1320
	TACCAACATG	TCCGGAGCCA	CATGGACAGA	GGCCGGCTCG	GCCCCACCCTC	TGCCCTGAGA	1380
	GTGACCGCTG	TACCAACCTC	TGTCCCTACA	GGGCAGCCCC	GAGAACCACA	GGTGACACC	1440
	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG	AACCAGGTCA	GCCTGACCTG	CCTGGTCAAA	1500
	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	1560
25	TACAAGACCA	CGCCTCCCGT	GCTGGACTCC	GACGGCTCCT	TCTTCTCTTA	CAGCAAGCTC	1620
	ACCGTGAGCA	AGAGCAGGTG	GCAGCAGGGG	AACGCTCTCT	CATGCTCCGT	GATGCATGAG	1680
	GCTCTGCACA	ACCCTACAC	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA	ATGAGTGCGA	1740
	CGGCCGGCAA	GCCCCCGCTC	CCCGGGCTCT	CGCGGTGCGA	CGAGGATGCT	TGGCACGTAC	1800
	CCCCTGTACA	TACTTCCCGG	GCGCCCAGCA	TGGAAATAAA	GCACCCAGCG	CTGCCCTGGG	1860
30	CCCCTGCGAG	ACTGTGATGG	TTCTTTCCAC	GGGTGAGGCC	GAGTCTGAGG	CCTGAGTGCC	1920
	ATGAGGGAGG	CAGAGCGGGT	CCCACTGTCC	CCACACTGGC	CCAGGCTGTG	CAGGTGTGCC	1980
	TGGGCCCCCT	AGGGTGGGGC	TCAGCCAGGG	GCTGCCCTCG	GCAGGGTGGG	GGATTTGCCA	2040
	GCGTGCCCTT	CCCTCCAGCA	GACCTGCCC	TGGGCTGGGC	CACGGGAAGC	CCTAGGAGCC	2100
	CCTGGGGACA	GACACACAGC	CCCTGCCTCT	GTAGGAGACT	GTCCTGTTCT	GTGAGCGCCC	2160
35	CTGTCTCTCC	GACCTCCATG	CCCACTCGGG	GGCATGCCTA	GTCCATGTGC	GTAGGGACAG	2220
	GCCCTCCCTC	ACCCATCTAC	CCCCACGGCA	CTAACCCCTG	GCTGCCCTGC	CCAGCCTCGC	2280
	ACCCGCATGG	GGACACAACC	GACTCCGGGG	ACATGCACCTC	TCGGGCCCTG	TGGAGGGACT	2340
	GGTGACAGATG	CCCACACACA	CACCTCAGCC	AGACCCGTTT	AACAAACCCC	GCACTGAGGT	2400
	TGGCCGGCCA	CACGGCCACC	ACACACACCC	GTGCACGCCT	CACACACGGA	GCCTCACCCG	2460
40	GGCGAACTGC	ACAGCACCCA	GACCAGAGCA	AGGTCTCTCG	ACACGTGAAC	ACTCCTCGGA	2520
	CACAGGCCCC	CACGAGCCCC	ACGCGGCACC	TCAAGGCCCA	CGAGCCTCTC	GGCAGCTTCT	2580
	CCACATGCTG	ACCTGCTCAG	ACAAACCCAG	CCCTCCTCTC	ACAAGGGTGC	CCCTGCAGCC	2640
	GCCACACACA	CACAGGGGAT	CACACACCAC	GTCACGTCCC	TGGCCCTGGC	CCACTTCCCA	2700
	GTGCCGCCCT	TCCCTGCAGG	ACGGATCAGC	CTCGACTGTG	CCTTCTAGTT	GCCAGCCATC	2760
45	TGTTGTTTGC	CCCTCCCCCG	TGCCTTCCTT	GACCCCTGAA	GGTGCCACTC	CCACTGTCTT	2820
	TTCTTAATAA	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT	CTATTCTGGG	2880
	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAATAGCA	GGCATGCTGG	2940
	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAACC	AGCTGGGGCT	CTAGGGGGTA	3000
	TCCCCACGCG	CCCTGTAGCG	GCGCATTAA	GCGGCGGGT	GTGGTGGTTA	CGCGCAGCGT	3060
50	GACCGCTACA	CTTGCCAGCG	CCCTAGCGCG	CGCTCCTTTC	GCTTCTTCTC	CTTCTTCTT	3120
	CGCCACGTTT	GCCGGGCCCT	TCAAAAAAGG	GAAAAAAGC	ATGCATCTCA	ATTAGTCAGC	3180
	AACCATAGTC	CCGCCCCCTA	CTCCGCCCAT	CCCGCCCCCTA	ACTCCGCCCA	GTTCGCCCCA	3240
	TTCTCCGCCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA	GAGGCCGAGG	CCGCCTCGGC	3300
	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG	CTTTTTTGGA	GGCCTAGGCT	TTTGCAAAAA	3360
55	GCTTGGACAG	CTCAGGGCTG	CGATTTGCGG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	3420
	CTGGTAGGAT	TTTATCCCCG	CTGCCATCAT	GGTTCGACCA	TTGAACTGCA	TCGTCGCGGT	3480
	GTCCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC	TCAGGAACGA	3540
	GTTCAAGTAC	TTCCAAAGAA	TGACCACAAC	CTCTTCAGTG	GAAGGTAAAC	AGAATCTGGT	3600
	GATTATGGGT	AGGAAAACCT	GGTTCTCCAT	TCCTGAGAAG	AATCGACCTT	TAAAGGACAG	3660

	AATTAATATA	GTTCTCAGTA	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	ATTTTCTTGC	3720
	CAAAAGTTTG	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	GTAAAGTAGA	3780
	CATGGTTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC	AACCAGGCCA	3840
	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTTGAA	AGTGACACGT	TTTCCCAGA	3900
5	AATTGATTTG	GGGAAATATA	AACTTCTCCC	AGAATACCCA	GGCGTCCTCT	CTGAGGTCCA	3960
	GGAGGAAAAA	GGCATCAAGT	ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	4020
	TGCTTTCAAG	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTATAAGAC	CATGGGACTT	4080
	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC	ATAATTGGAC	4140
	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA	AATTTTAAAG	TGTATAATGT	4200
10	GTAAACTAC	TGATTCTAAT	TGTTTGTGTA	TTTTAGATTG	CAACCTATGG	AACTGATGAA	4260
	TGGGAGCAGT	GGTGGAATGC	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	AGAAATGCCA	4320
	TCTAGTGATG	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	AAAGAAGAGA	4380
	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTTGAG	TCATGCTGTG	4440
	TTTAGTAATA	GAACTCTTGC	TTGCTTTGCT	ATTTACACCA	CAAAGGAAAA	AGCTGCACTG	4500
15	CTATACAAGA	AAATTATGGA	AAAATATTCT	GTAACCTTTA	TAAGTAGGCA	TAACAGTTAT	4560
	AATCATAACA	TACTGTTTTT	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	4620
	TATGCTCAAA	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTTAA	TAAGGAATAT	4680
	TTGATGTATA	GTGCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTTG	TAGAGGTTTT	4740
	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA	TGAATGCAAT	4800
20	TGTTTGTGTT	AACTTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA	ATAGCATCAC	4860
	AAATTTACCA	AATAAAGCAT	TTTTTTCATT	GCATTCTAGT	TGTGGTTTGT	CCAAACTCAT	4920
	CAATGTATCT	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	ATCTCATGCT	4980
	GGAGTTCTTC	GCCCAACCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA	AATAAAGCAA	5040
	TAGCATCACA	AATTTACAAA	ATAAAGCATT	TTTTTCACTG	CATTCTAGTT	GTGGTTTGTC	5100
25	CAAACCTATC	AATGTATCTT	ATCATGTCTG	TATACCGTCG	ACCTCTAGCT	AGAGCTTGCG	5160
	GTAATCATGG	TCATAGCTGT	TTCCTGTGTG	AAATGTTTAT	CCGCTCACAA	TCCACACAA	5220
	CATACGAGCC	GGAAAGCATA	AGTGTAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAATCTAC	5280
	ATTAATTGCG	TTGCGCTCAC	TGCCCCGTTT	CCAGTCGGGA	AACCTGTCTG	GCCAGCTGCA	5340
	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCCT	ATTGGGCGCT	CTTCCGCTTC	5400
30	CTCGCTCACT	GACTCGCTGC	GCTCGGTCGT	TCCGCTGCGG	CGAGCGGTAT	CAGCTCACTC	5460
	AAAGGCGGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	5520
	AAAAGGCCAG	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	TTTTCCATAG	5580
	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TGCACGCTCA	AGTCAGAGGT	GGCGAAACCC	5640
	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC	TCCTCTGTGC	GCTCTCCTGT	5700
35	TCCGACCCCTG	CCGCTTACCG	GATACCTGTC	CGCCTTTCTC	CCTTCGGGAA	GCGTGGCGCT	5760
	TTCTCAATGC	TCACGCTGTA	GGTATCTCAG	TTCGTTGTAG	GTGTTTCGCT	CCAAGCTGGG	5820
	CTGTGTGCAC	GAACCCCCCG	TTCAGCCCCG	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT	5880
	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	GTAACAGGAT	5940
	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGCC	CTAACTACGG	6000
40	CTACAGTAGA	AGGACAGTAT	TTGGTTACTG	CGTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	6060
	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAAACA	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	6120
	TTGCAAGCAG	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	6180
	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCAGCTTAA	GGGATTTTGG	TCATGAGATT	6240
	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAATAA	TGAAGTTTAA	AATCAATCTA	6300
45	AAGTATATAT	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	6360
	CTCAGCGATC	TGTCTATTTT	GTTTATCCAT	AGTTGCCTGA	CTCCCCGTGC	TGTAGATAAC	6420
	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA	ATGATACCGC	GAGACCCACG	6480
	CTCACC GGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG	AGCGCAGAAG	6540
	TGGTCCTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	6600
50	AAGTAGTTCC	CCAGTTAATA	GTTTGCAGCA	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	6660
	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGTCCGGT	TCCCAACGAT	CAAGGCGAGT	6720
	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GCTTAGCTCC	TTCGGTCCCTC	CGATCGTTGT	6780
	CAGAAGTAAG	TTGGCCGCGAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC	ATAATTCTCT	6840
	TACTGT CATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	6900
55	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCG	GCGTCAATAC	GGGATAATAC	6960
	CGCGCCACAT	AGCAGAACTT	TAAAAGTGCT	CATCATTTGA	AAACGTTCTT	CGGGGCGAAA	7020
	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTCGATG	TAACCCACTC	GTGCACCCAA	7080
	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG	CGTTTCTGGG	TGAGCAAAAA	CAGGAAGGCA	7140
	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	TACTCTTCTT	7200

TTTTCAATAT TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTGA 7260
 ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC 7320
 TGACGTCGAC GGATCGGGAG ATCTGCTAGG TGACCTGAGG CGCGCCGGCT TCGAATAGCC 7380
 AGAGTAACCT TTTTTTTTAA TTTTATTTTA TTTTATTTTT GAGATGGAGT TTGGCGCCGA 7440
 5 TCTCCCGATC CCCTATGGTC GACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC 7500
 CAGTATCTGC TCCCTGCTTG TGTGTTGGAG GTCGCTGAGT AGTGCGCGAG CAAAATTTAA 7560
 GCTACAACAA GGCAAGGCTT GACCGACAAT TGCATGAAGA ATCTGCTTAG GGTAGGCGT 7620
 TTTGCGCTGC TTGCGCATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT 7680
 TATTAATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT 7740
 10 ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCGG CCCATTGACG 7800
 TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG 7860
 GTGGACTATT TACGGTAAAC TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT 7920
 ACGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCCCCTT GGCATTATGC CCAGTACATG 7980
 ACCTTAATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG 8040
 15 GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGA CTC ACGGGGATT 8100
 CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGT TTTT GGCACCAAAA TCAACGGGAC 8160
 TTTCCAAAT GTCGTAACAA CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGGTACGG 8220
 TGGGAGGTCT ATATAAGCAG AGCTCTCTGG CTAAC TAGAG AACCCACTGC TTACTGGCTT 8280
 ATCGAAATTA ATACGACTCA CTATAGGGAG ACCCAAGCTT G 8321
 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 8897 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GACGGATCGG GAGATCTGCT AGCCCGGGTG ACCTGAGGCG CGCCGGCTTC GAATAGCCAG 60
 AGTAACCTTT TTTTTTAATT TTATTTTATT TTATTTTGA GATGGAGTTT GGCGCCGATC 120
 35 TCCCGATCCC CTATGGTCGA CTCTCAGTAC AATCTGCTCT GATGCCGCAT AGTTAAGCCA 180
 GTATCTGCTC CCTGCTTG TGTTGGAGGT CGCTGAGTAG TGCGCGAGCA AAATTTAAGC 240
 TACAACAAGG CAAGGCTTGA CCGACAATTG CATGAAGAAT CTGCTTAGGG TTAGGCGTTT 300
 TGCCTGCTT CGCGATGTAC GGGCCAGATA TACGCGTTGA CATTGATTAT TGACTAGTTA 360
 TTAATAGTAA TCAATTACGG GGTCAATTAGT TCATAGCCCA TATATGGAGT TCCGCGTTAC 420
 40 ATAACCTACG GTAAATGGCC CGCCTGGCTG ACCGCCCAAC GACCCCGGCC CATTGACGTC 480
 AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC GTCAATGGGT 540
 GGACTATTTA CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATCATA TGCCAAGTAC 600
 GCCCCCTAT TACGTCAATG ACGGTAAATG GCCCGCTGG CATTATGCCC AGTACATGAC 660
 CTTATGGGAC TTTCCTACTT GGCAGTACAT CTACGTATTA GTCATCGCTA TTACCATGGT 720
 45 GATGCGGTTT TGGCAGTACA TCAATGGGCG TGGATAGCGG TTTGACTCAC GGGGATTTC 780
 AAGTCTCCAC CCCATTGACG TCAATGGGAG TTTGTTTGG CACCAAAATC AACGGGACTT 840
 TCCAAATGT CGTAACAAC CCGCCCCATT GACGCAAATG GGCGGTAGGC GTGTACGGTG 900
 GGAGGTCTAT ATAAGCAGAG CTCTCTGGCT AACTAGAGAA CCCACTGCTT ACTGGCTTAT 960
 CGAAATTAAT ACGACTCACT ATAGGGAGAC CCAAGCTTGG TACCAATTTA AATTGATATC 1020
 50 TCCTTAGGTC TCGAGCACCA TGAAGTTGCC TGTTAGGCTG TTGGTGCTGA TGTTCTGGAT 1080
 TCCTGCTTCC AGCAGTGATG TTGTCATGAC CCAAAACCCA CTGTCCAGTC CTGTACGCT 1140
 TGGACAACCT GCGTCCATCT CTTGCAGATC TAGTCAGATC ATTGTACATA ATAATGGCAA 1200
 CACCTATCTG GAATGGTACC AGCAGAGACC AGGGCAGTCT CCACGGCTCC TGATCTACAA 1260
 AGTTTCCAAC CGATTTTCTG GGGTCCCAGA CAGGTTGAGC GGCAGTGGAG CTGGGACAGA 1320
 55 TTTCACACTC AAGATCAGCA GAGTGGAGGC TGAGGATGTG GGAGTTTACT ACTGCTTCCA 1380
 GGGTTCACAT GTTCCATTCA CGTTCGGCCA AGGGACAAAG TTGGAAATCA AACGTAAGTC 1440
 TCGAGTCTCT AGATAACCGG TCAATCGATT GGAATTCTAA ACTCTGAGGG GGTCCGATGA 1500
 CGTGCCCAT TTTGCTTAA AGCATTGAGT TTAGTGCAAG GTCAGAAAAG CATGCAAAGC 1560
 CCTCAGAATG GCTGCAAAGA GCTCCAACAA AACAAATTTAG AACTTTTATTA AGGAATAGGG 1620

	GGAAGCTAGG	AAGAAACTCA	AAACATCAAG	ATTTTAAATA	CGCTTCTTGG	TCTCCTTGCT	1680
	ATAATTATCT	GGGATAAGCA	TGCTGTTTTT	TGTCTGTCCC	TAACATGCCC	TTATCCGCAA	1740
	ACAACACACC	CAAGGGCAGA	ACTTTGTTAC	TTAAACACCA	TCCTGTTTGC	TTCTTTCCTC	1800
	AGGAACTGTG	GCTGCACCAT	CTGTCTTCAT	CTTCCCGCCA	TCTGATGAGC	AGTTGAAATC	1860
5	TGGAAC TGCC	TCTGTTGTGT	GCCTGCTGAA	TAACTTCTAT	CCCAGAGAGG	CCAAAGTACA	1920
	GTGGAAGGTG	GATAACGCCC	TCCAATCGGG	TAACTCCCAG	GAGAGTGTCA	CAGAGCAGGA	1980
	GAGCAAGGAC	AGCACCTACA	GCCTCAGCAG	CACCCTGACG	CTGAGCAAAG	CAGACTACGA	2040
	GAAACACAAA	GTCTACGCCT	GCGAAGTCAC	CCATCAGGGC	CTGAGCTCGC	CCGTCACAAA	2100
	GAGCTTCAAC	AGGGGAGAGT	GTTAGAGGGA	GAAGTGCCCC	CACCTGCTCC	TCAGTTCCAG	2160
10	CCTGACCCCC	TCCCATCCTT	TGGCCTCTGA	CCCTTTTTCC	ACAGGGGACC	TACCCCTATT	2220
	GCGGTCTCTC	AGCTCATCTT	TCACCTCACC	CCCTCCTCC	TCCTTGCGTT	TAATTATGCT	2280
	AATGTTGGAG	GAGAATGAAT	AAATAAAGTG	AATCTTTGCA	CCTGTGGTTT	CTCTCTTTCC	2340
	TCATTTAATA	ATTATTATCT	GTTGTTTTAC	CACTACTCA	ATTCTCTTTA	TAAGGGACTA	2400
	AATATGTAGT	CATCCTAAGG	CACGTAACCA	TTTATAAAAA	TCATCCTTCA	TTCTATTTTA	2460
15	CCCTATCATC	CTCTGCAAGA	CAGTCCTCCC	TCAAACCCAC	AAGCCTTCTG	TCCTCACAGT	2520
	CCCCTGGGCC	ATGGTAGGAG	AGACTTGCTT	CCTTGTTTTT	CCCTCCTCAG	CAAGCCCTCA	2580
	TAGTCCTTTT	TAAGGGTGAC	AGGTCTTACA	GTCATATATC	CTTTGATTCA	ATTCCCTGAG	2640
	AATCAACCAA	AGCAAATTTT	TCAAAAGAAG	AAACCTGCTA	TAAAGAGAAT	CATTCAATTG	2700
	AACATGATAT	AAAATAACAA	CACAATAAAA	GCAATTAAT	AAACAAACAA	TAGGGAAATG	2760
20	TTTAAGTTCA	TCATGGTACT	TAGACTTAAT	GGAATGTCTA	GCCTTATTTA	CATTTTTTAA	2820
	CAGGTACTGA	GGGACTCCTG	TCTGCCAAGG	GCCGTATTGA	GTACTTTCCA	CAACCTAATT	2880
	TAATCCACAC	TATACTGTGA	GATTAAAAAC	ATTCATTAAA	ATGTTGCAAA	GGTTCATAAA	2940
	AGCTGAGAGA	CAAATATATT	CTATAACTCA	GCAATCCAC	TTCTAGATGA	CTGAGTGTCC	3000
	CCACCCACCA	AAAAACTATG	CAAGAATGTT	CAAAGCAGCT	TTATTTACAA	AAGCCAAAAA	3060
25	TTGGAAATAG	CCCGATTGTC	CAACAATAGA	ATGAGTTATT	AACTGTGGT	ATGTTTATAC	3120
	ATTAGAATAC	CCAATGAGGA	GAATTAACAA	GCTACCACTA	TACCTACTCA	CACAGATGAA	3180
	TCTCATAAAA	ATAATGTTAC	ATAAGAGAAA	CTCAATGCAA	AAGATATGTT	CTGTATGTTT	3240
	TCATCCATAT	AAAGTTCAAA	ACCAGGTAAA	AATAAAGTTA	GAAATTTGGA	TGGAAATTAC	3300
	TCTTAGCTGG	GGGTGGGCGA	GTTAGTGCCT	GGGAGAAGAC	AAGAAGGGGC	TTCTGGGGTC	3360
30	TTGGTAATGT	TCTGTTCCCT	GTGTGGGGTT	GTGCAAGTTA	GATCTGTGCA	CTGTTCTGTA	3420
	TACACATTAT	GCTTCAAAAT	AACTTCACAT	AAAGAACATC	TTATACCCAG	TTAATAGATA	3480
	GAGAGGAAT	AAGTAATAGG	TCAAGACCAA	CGCAGCTGGT	AAGTGGGGGC	CTGGGATCAA	3540
	ATAGCTACCT	CCCTAATCCT	GCCCWCTTGA	GCCCTGAATG	AGTCTGCCTT	CCAGGGCTCA	3600
	AGGTGCTCAA	CAAAACAACA	GGCCTGCTAT	TTTCTGGCA	TCTGTGCCCT	GTTTGGCTAG	3660
35	CTAGGAGCAC	ACATACATAG	AAATTAATG	AAACAGACCT	TCAGCAAGGG	GACAGAGGAC	3720
	AGAATTAACC	TTGCCCAGAC	ACTGGAACCC	CATGTATGAA	CACTCACATG	TTTGGGAAGG	3780
	GGGAAGGGCA	CATGTAAATG	AGGACTCTTC	CTCATTCTAT	GGGGCACTCT	GGCCCTGCCC	3840
	CTCTCAGTCA	CTCATCCATC	CAACACACCT	CTCTAAGTAC	CTCTCTCTGC	CTACACTCTG	3900
	AAGGGGTTCA	GGAGTAACTA	ACACAGCATC	CTTTCCTCA	AATGACTGAC	AATCCCTTTG	3960
40	TCCTGCTTTG	TTTTTCTTTT	CAGTCAGTAC	TGGGAAAGTG	GGGAAGGACA	GTCATGGAGA	4020
	AACTACATAA	GGAAGCACCT	TGCCCTTCTG	CCTCTTGAGA	ATGTTGATGA	GTATCAAATC	4080
	TTTCAAACCT	TGGAGGTTTG	AGTAGGGGTG	AGACTCAGTA	ATGTCCCTTC	CAATGACATG	4140
	AACTTGCTCA	CTCATCCCTG	GGGGCCAAAT	TGAACAAATC	AAGGCAGGCA	TAATCCAGTT	4200
	ATGAATTCTT	GCGGCCGCTT	GCTAGCTTCA	CGTGTGGAT	CCAACCGCGG	AAGGGCCCTA	4260
45	TTCTATAGTG	TCACCTAAAT	GCTAGAGCTC	GCTGATCAGC	CTCGACTGTG	CCTTCTAGTT	4320
	GCCAGCCATC	TGTTGTTTGC	CCCTCCCCCG	TGCCCTCCTT	GACCCTGGAA	GGTGCCACTC	4380
	CCACTGTCTT	TTCCCTAATA	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT	4440
	CTATTCTGGG	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAAATAGCA	4500
	GGCATGCTGG	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAACC	AGCTGGGGCT	4560
50	CTAGGGGGTA	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAG	CGCGGCGGGT	GTGGTGGTTA	4620
	CGCGCAGCGT	GACCGCTACA	CTTGCCAGCG	CCCTAGCGCC	CGCTCCTTTC	GCTTTCTTCC	4680
	CTTCTTTTCT	CGCCACGTTT	GCCGGGCCTC	TCAAAAAAGG	GAAAAAAGC	ATGCATCTCA	4740
	ATTAGTCAGC	AACCATAGTC	CCGCCCTTAA	CTCCGCCCAT	CCCGCCCTTA	ACTCCGCCCA	4800
	GTTCCGCCCA	TTCTCCGCCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA	GAGGCCGAGG	4860
55	CCGCCTCGGC	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG	CTTTTTTGGA	GGCCTAGGCT	4920
	TTTGCAAAAA	GCTTGACAG	CTCAGGGCTG	CGATTTTCGG	CCAAACTTGA	CGGCAATCCT	4980
	AGCGTGGAAG	CTGGTAGGAT	TTTATCCCCG	CTGCCATCAT	GGTTTCGACCA	TTGAATCGCA	5040
	TCGTGCGCGT	TGCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC	5100
	TCAGGAACGA	GTTCAAGTAC	TTCCAAAGAA	TGACCACAAC	CTCTTCAGTG	GAAGGTAAAC	5160

	AGAATCTGGT	GATTATGGGT	AGGAAAACCT	GTTTCTCCAT	TCCTGAGAAG	AATCGACCTT	5220
	TAAAGGACAG	AATTAATATA	GTTCTCAGTA	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	5280
	ATTTTCTTGC	CAAAAGTTTG	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	5340
	GTAAAGTAGA	CATGGTTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC	5400
5	AACCAGGCCA	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTTGAA	AGTGACACGT	5460
	TTTTCCCAGA	AATTGATTTG	GGGAAATATA	AACTTCTCCC	AGAATACCCA	GGCGTCTCT	5520
	CTGAGGTCCA	GGAGGAAAAA	GGCATCAAGT	ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	5580
	AACAGGAAGA	TGCTTTCAAG	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	5640
	CATGGGACTT	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC	5700
10	ATAATTGGAC	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA	AATTTTTAAG	5760
	TGTATAATGT	GTTAAACTAC	TGATTCTAAT	TGTTTGTGTA	TTTTAGATTG	CAACCTATGG	5820
	AACTGATGAA	TGGGAGCAGT	GGTGGAATGC	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	5880
	AGAAATGCCA	TCTAGTGATG	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	5940
	AAAGAAGAGA	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTTGAG	6000
15	TCATGCTGTG	TTTAGTAATA	GAACCTTGC	TTGCTTTGCT	ATTTACACCA	CAAAGGAAAA	6060
	AGCTGCACTG	CTATACAAGA	AAATTATGGA	AAAATATTCT	GTAACCTTTA	TAAGTAGGCA	6120
	TAACAGTTAT	AATCATAACA	TACTGTTTTT	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	6180
	TATTAATAAC	TATGCTCAAA	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTAA	6240
	TAAGGAATAT	TTGATGTATA	GTGCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTTG	6300
20	TAGAGGTTTT	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA	6360
	TGAATGCAAT	TGTTGTTGTT	AACCTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA	6420
	ATAGCATCAC	AAATTCACA	AATAAAGCAT	TTTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	6480
	CCAAACTCAT	CAATGTATCT	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	6540
	ATCTCATGCT	GGAGTTCTTC	GCCCACCCCA	ACTTGTTTTAT	TGCAGCTTAT	AATGGTTACA	6600
25	AATAAAGCAA	TAGCATCACA	AATTTACAAA	ATAAAGCATT	TTTTTCACTG	CATTCTAGTT	6660
	GTGGTTTGTC	CAAACTCATC	AATGTATCTT	ATCATGTCTG	TATACCGTCG	ACCTCTAGCT	6720
	AGAGCTTGGC	GTAATCATGG	TCATAGCTGT	TTCTGTGTG	AAATTGTTAT	CCGCTCACAA	6780
	TTCCACACAA	CATACGAGCC	GGAAGCATAA	AGTGTAAGC	CTGGGGTGCC	TAATGAGTGA	6840
	GCTAACTCAC	ATTAATTGCG	TTGCGCTCAC	TGCCCCGCTT	CCAGTCGGGA	AACCTGTCTG	6900
30	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCGT	ATTGGGCGCT	6960
	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	GCTCGGTCGT	TCGGCTGCGG	CGAGCGGTAT	7020
	CAGCTCACTC	AAAGGCGGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	7080
	ACATGTGAGC	AAAAGGCCAG	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	7140
	TTTTCCATAG	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT	7200
35	GGCGAAACCC	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC	TCCTCGTGTC	7260
	GCTCTCCTGT	TCCGACCCTG	CCGCTTACCG	GATACCTGTC	CGCCTTTCTC	CCTTCGGGAA	7320
	GCGTGCGCGT	TTCTCAATGC	TCACGCTGTA	GGTATCTCAG	TTCGGTGTAG	GTCGTTGCTG	7380
	CCAAGCTGGG	GTGTGTGCAC	GAACCCCGCG	TTACGCCCCG	CCGCTGCGCC	TTATCCGGTA	7440
	ACTATCGTCT	TGAGTCCAAC	CCGGTAAGAC	GCAGCTTATC	GCCACTGGCA	GCAGCCACTG	7500
40	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC	7560
	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	7620
	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	7680
	GTTTTTTTGT	TTGCAAGCAG	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	7740
	TGATCTTTTC	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	7800
45	TCATGAGATT	ATCAAAAAGG	ATCTTACCTT	AGATCCTTTT	AAATTAATAA	TGAAGTTTGA	7860
	AATCAATCTA	AAGTATATAT	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	7920
	AGGCACCTAT	CTCAGCGATC	TGTCTATTTT	GTTTATCCAT	AGTTGCCTGA	CTCCCCGTCG	7980
	TGTAGATAAC	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA	ATGATACCGC	8040
	GAGACCCACG	CTCACC GGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG	8100
50	AGCGCAGAAG	TGGTCTTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	8160
	AAGCTAGAGT	AAGTAGTTTC	CCAGTTAATA	GTTTGC GCAA	CGTTGTTGCC	ATTGCTACAG	8220
	GCATCGTGGT	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	8280
	CAAGGCGAGT	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GGTAGCTCC	TTCGGTCTCT	8340
	CGATCGTTGT	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC	8400
55	ATAATTCTCT	TACTGTCAAT	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	8460
	CCAAGTCATT	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCG	GCGTCAATAC	8520
	GGGATAATAC	CGCGCCACAT	AGCAGAACTT	TAAAAGTGCT	CATCATTGGA	AAACGTTCTT	8580
	CGGGCGGAAA	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTTCGATG	TAACCCACTC	8640
	GTGCACCCAA	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG	CGTTTCTGGG	TGAGCAAAAA	8700

CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	8760
TACTCTTCCT	TTTTCAATAT	TATTGAAGCA	TTATCAGGG	TTATTGTCTC	ATGAGCGGAT	8820
ACATATTTGA	ATGTATTTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTCCCCGAA	8880
AAGTGCCACC	TGACGTC					8897

What is claimed is:

1. A method for inhibiting immunoglobulin-induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering an immunoglobulin molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited.
2. A method for inhibiting immunoglobulin-induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering a structurally altered antibody to the subject, the structurally altered antibody comprising a variable region and a constant region, multiple toxicity associated domains in the constant region being modified so as to render the constant region unable to mediate an ADCC response or activate complement thereby inhibiting immunoglobulin-induced toxicity resulting from immunotherapy.
3. A method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an Ig fusion protein to the subject, the Ig fusion protein having multiple structurally altered toxicity associated domains in the constant region.
4. A method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an Ig fusion protein to the subject, the Ig fusion protein comprising a modified constant region, the

modification being a structural alteration in multiple toxicity associated regions within the CH₂ domain.

5. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
- (a) selecting an immunoglobulin which recognizes and binds a target, the target being associated with the disease;
- (b) mutating the immunoglobulin so selected by structurally altering multiple toxicity associated domains in the constant region of the immunoglobulin thereby creating a structurally altered immunoglobulin;
- (c) administering the structurally altered immunoglobulin of step (b) to the subject under conditions so that the structurally altered immunoglobulin recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the constant region thereby preventing immunoglobulin-induced toxicity in the subject.
6. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
- (a) selecting an Ig fusion protein which recognizes and binds a target, the target being associated with the disease;
- (b) structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the Ig protein so selected;

- (c) administering the structurally altered Ig fusion protein of step (b) to the subject under conditions so that the structurally altered Ig fusion protein recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the CH₂ domain thereby preventing immunoglobulin-induced toxicity in the subject.
- 5
7. The method of claim 1, 2, 3, 4, 5, or 6, wherein the portion of the constant region is the CH₂ domain.
- 10
8. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgG.
9. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgM.
- 15
10. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgA.
11. The method of claim 2, wherein the antibody recognizes and binds Le^y.
- 20
12. The method of claim 2, wherein the antibody recognizes and binds to Le^x.
13. The method of claim 2, wherein the antibody is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
- 25
14. The method of claim 2, wherein the antibody is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.

15. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds Le^y.
16. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds to Le^x.
17. The method of claim 1 or 5, wherein the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
18. The method of claim 1 or 5, wherein the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
19. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds Le^y.
20. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds to Le^x.
21. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
22. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
23. A pharmaceutical composition comprising a pharmaceutically effective

amount of a structurally altered immunoglobulin, and an acceptable carrier, the structurally altered immunoglobulin (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.

- 5 24. A pharmaceutical composition comprising a pharmaceutically effective amount of structurally altered Ig fusion protein, and an acceptable carrier, the structurally altered Ig fusion protein (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.
- 10 25. A method of treating carcinomas in vivo comprising administering to a subject a pharmaceutically effective amount of the composition of claim 23 or 24.
- 15 26. The method of claim 30, wherein the structurally altered immunoglobulin in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- 20 27. The method of claim 24, wherein the Ig fusion protein in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- 25 28. The method of claim 2 or 5, wherein the antibody is conjugated to a cytotoxic agent.
29. The method of claim 1, wherein the immunoglobulin is conjugated to a cytotoxic agent.

30. The method of claim 3, 4 or 6, wherein the Ig fusion protein is conjugated to a cytotoxic agent.
31. The method of claim 28, 29, or 31, wherein the cytotoxic agent is selected
5 from the group consisting of antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents.
32. A method for treating a subject suffering from a cancer, the cancer being
10 characterized as a group of cells having a tumor associated antigen on the cell surface, which method comprises administering to the subject a cancer killing amount of the composition of claim 23 or 24 joined to a cytotoxic agent under conditions which permit the molecule so joined to bind the tumor associated antigen on the cell surface so as to kill the cells so bound
15 thereby curing the subject.
33. A pharmaceutical composition comprising a pharmaceutically effective amount of a structurally altered BR96 antibody, the structurally altered antibody having an inactivated CH₂ domain.
20
34. A method for treating a subject suffering from a proliferative type disease characterized by cells having a BR96 antigen on the cell surface which comprises administering to the subject an effective amount of the composition of claim 33 joined to doxorubicin such that the
25 immunoconjugate binds the BR96 antigen and kills said cells thereby treating the subject.
35. A method for inhibiting BR96 (ATCC: HB10036) induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering

BR96 to the subject, the BR96 molecule being modified prior to administration, the modification comprising the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated toxicity is inhibited.

36. A method for preventing BR96 (ATCC: HB10036) induced toxicity resulting from immunotherapy for cancer in a subject comprising:

(a) mutating the BR96 polypeptide by the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated immunoglobulin-induced toxicity is inhibited in the altered BR96 polypeptide; and

(b) administering the structurally altered BR96 polypeptide of step (a) to the subject under conditions so that the peptide recognizes and binds cancer associated Le^y antigens, thereby alleviating symptoms associated with the cancer, the structural alteration of the toxicity associated domains thereby preventing BR96 toxicity in the subject.

37. A chimeric BR96 antibody having a structurally altered constant region having the CH1 and CH3 domains but not the CH2 domain, the antibody being designated cBR96-A.

38. The chimeric BR96 antibody of claim 37 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 10.
39. A BR96 antibody having humanized variable and constant regions, wherein
5 the constant region has been structurally altered so that the CH1 and CH3 domains are present but the CH2 domain is not, the antibody being designated hBR96-2A.
40. The BR96 antibody of claim 39 which is expressed by the plasmid having
10 the sequence shown in SEQ ID NO. 12.
41. A BR96 antibody designated hBR96-2B having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine and glycine at amino acid position 237 is mutated to alanine.
15
42. A BR96 antibody designated hBR96-2C having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.
20
43. A BR96 antibody designated hBR96-2D having a structurally altered constant region wherein proline at amino acid position 331 is mutated to alanine.
- 25 44. A BR96 antibody designated hBR96-2E having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid

position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.

- 5 45. A BR96 antibody designated hBR96-2F having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; and proline at amino acid position 331 is mutated to alanine.
- 10 46. A BR96 antibody designated hBR96-2G having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
- 15 47. A BR96 antibody designated hBR96-2H having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
- 20
48. A nucleic acid molecule which encodes the BR96 antibody of claim 37, 39, and 41-47.
- 25 49. A cDNA of claim 48.
50. A plasmid which comprises the nucleic acid molecule of claim 48.

51. A host vector system comprising a plasmid of claim 50 in a suitable host cell.
52. A method for producing a protein comprising growing the host vector system of claim 51 so as to produce the protein in the host and recovering the protein
- 5 so produced.

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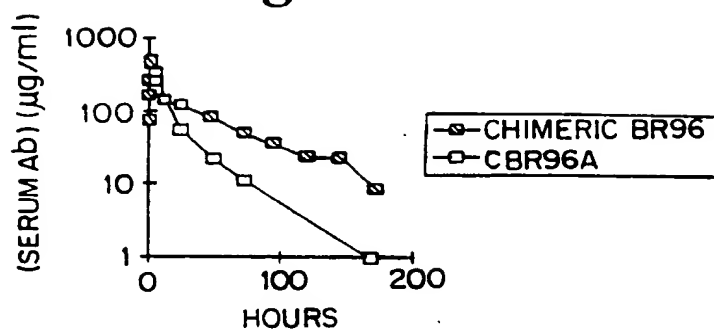
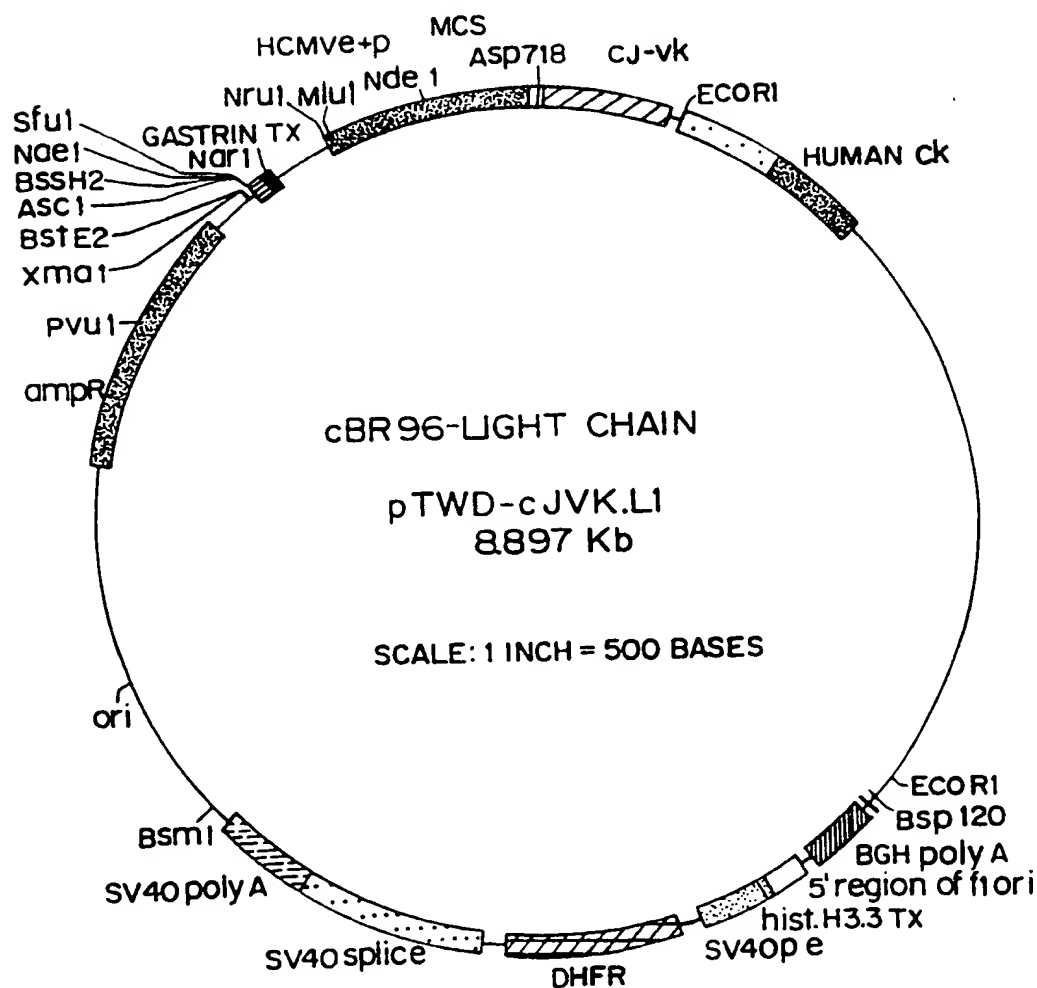
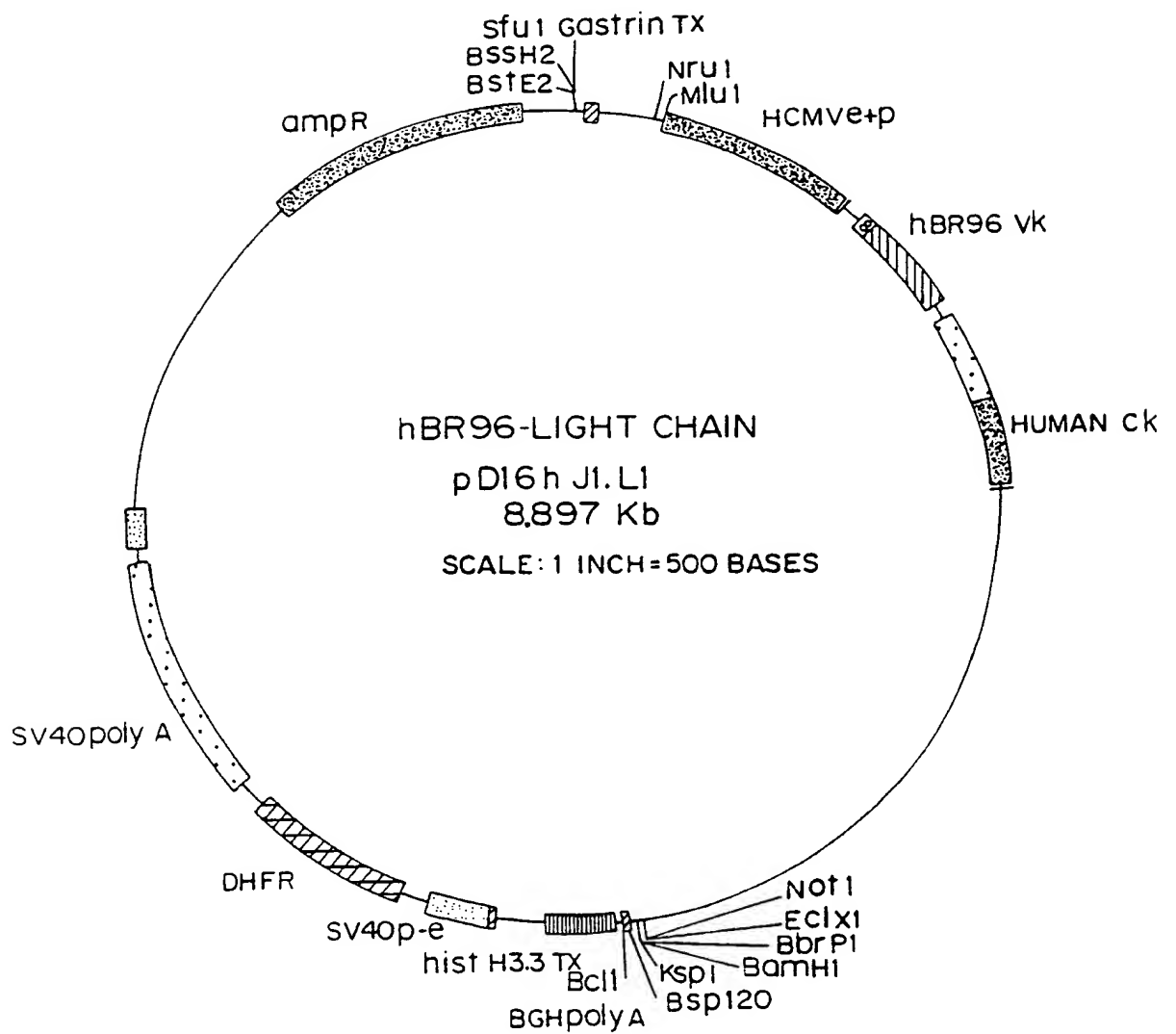
Fig. 1*Fig. 2*

Fig. 3

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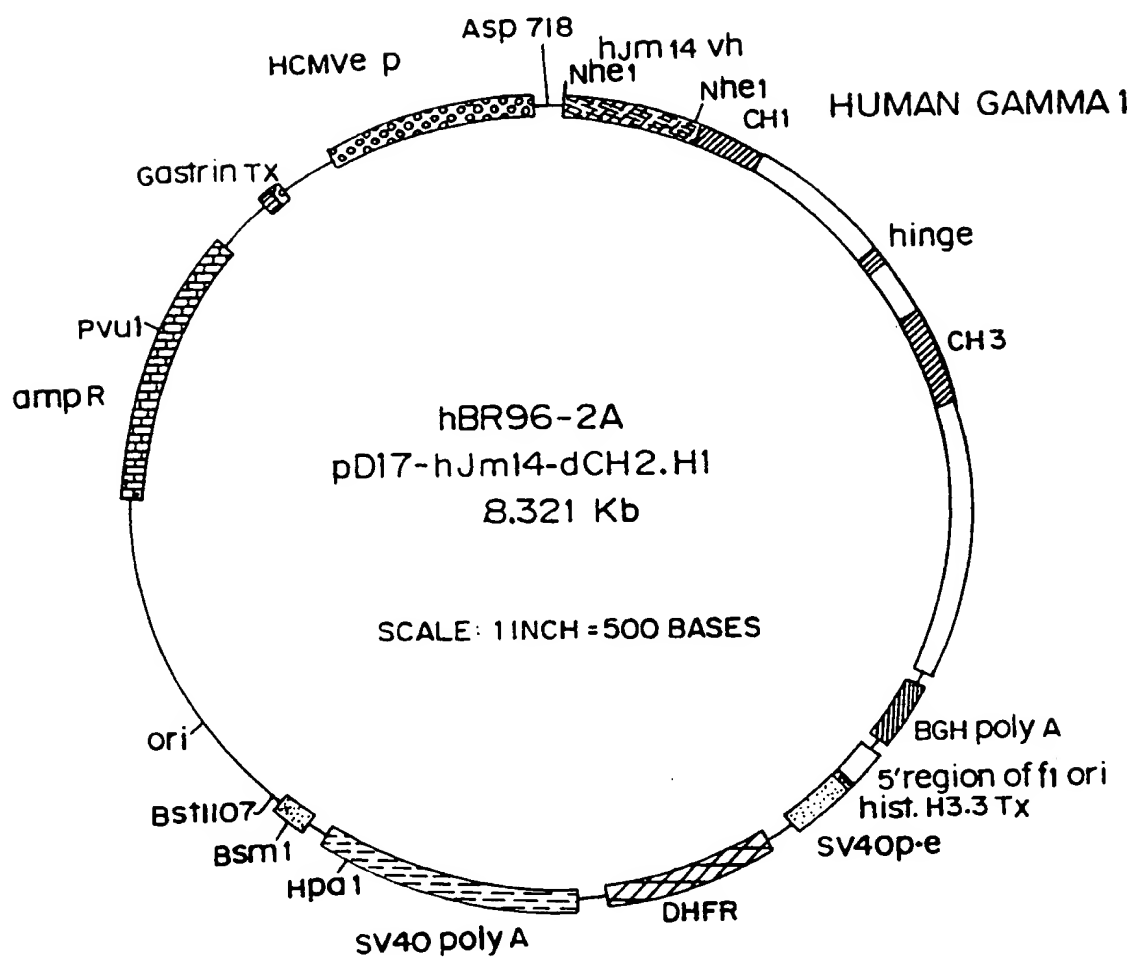
Fig. 4

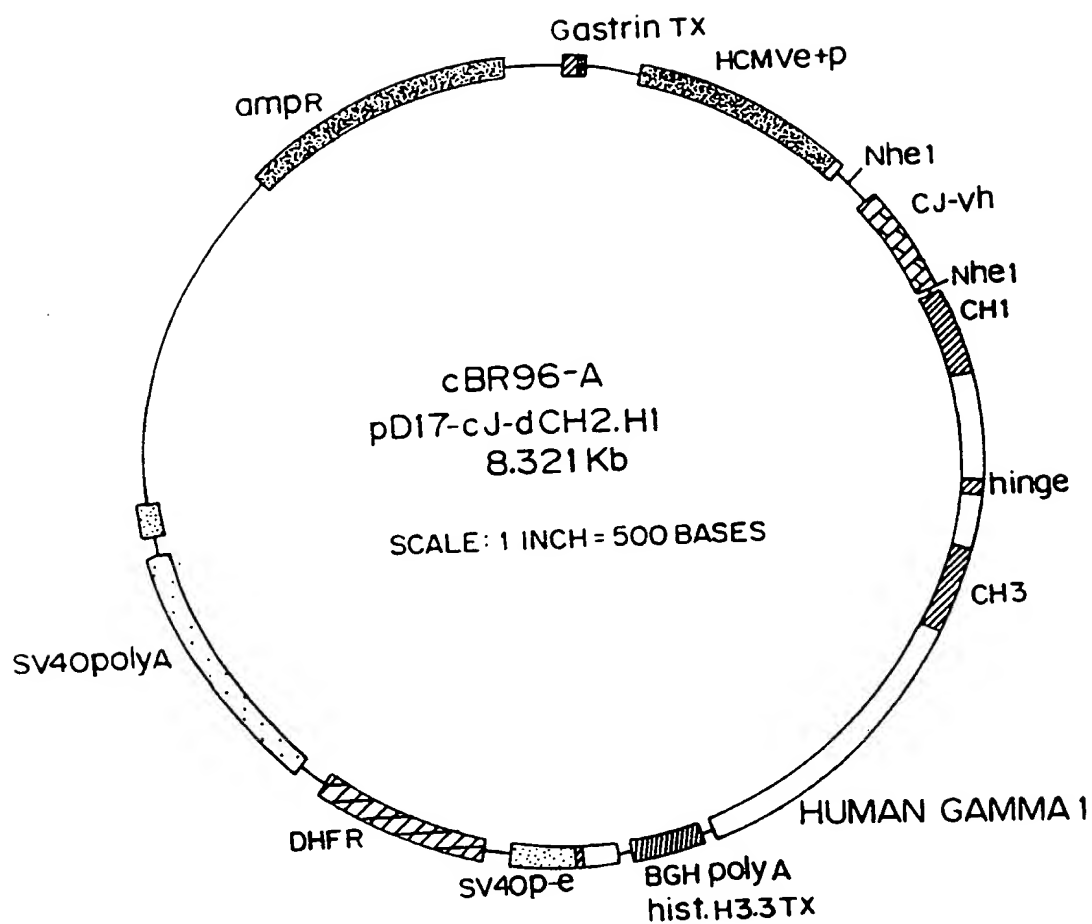
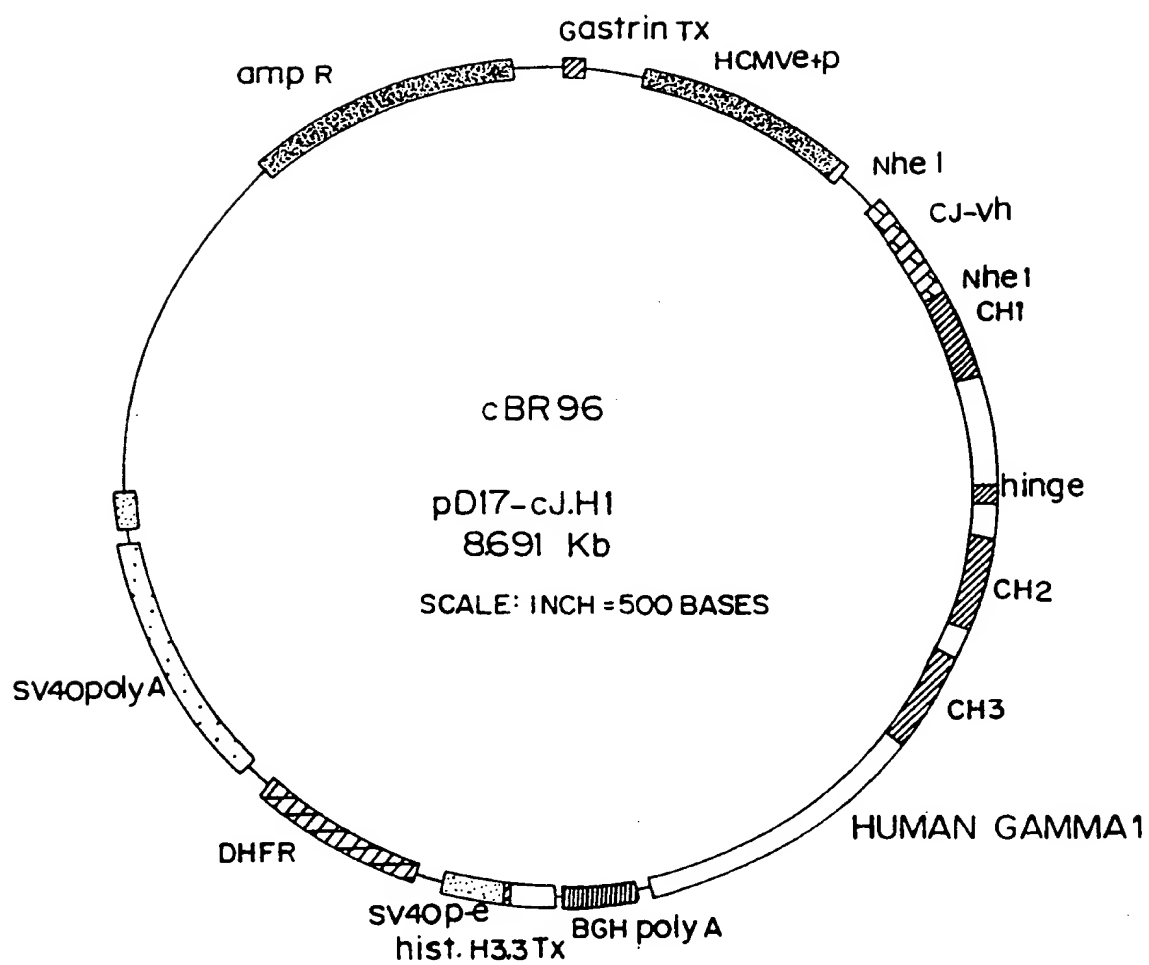
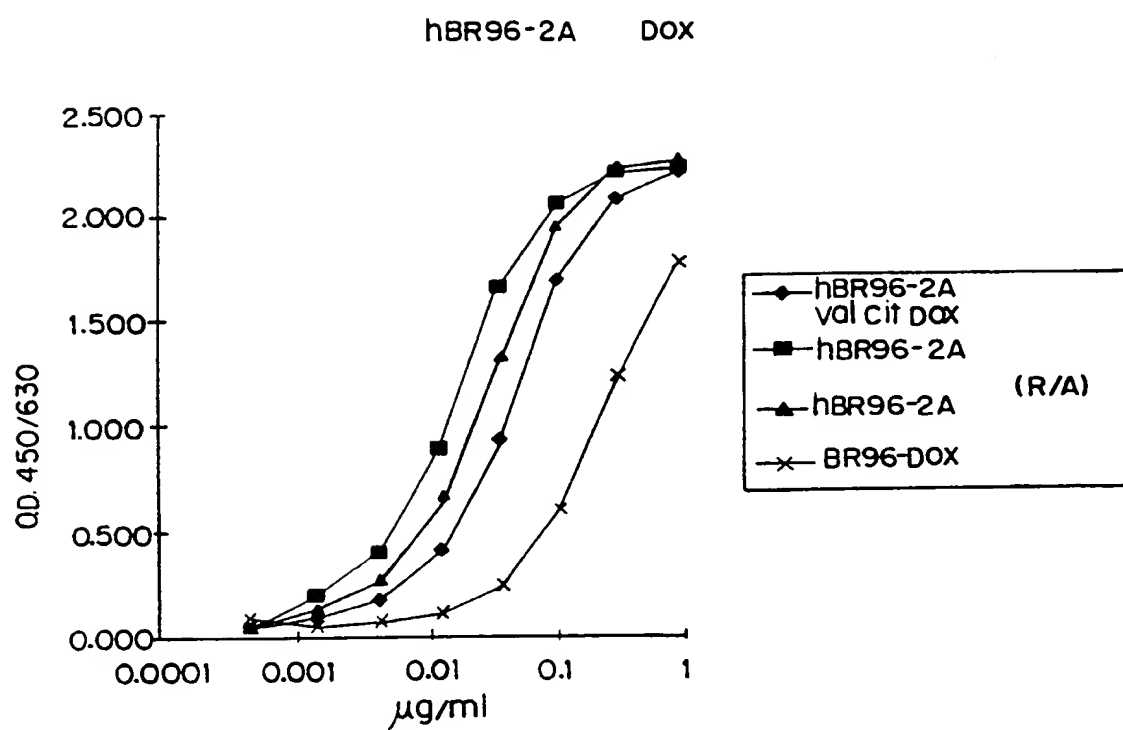
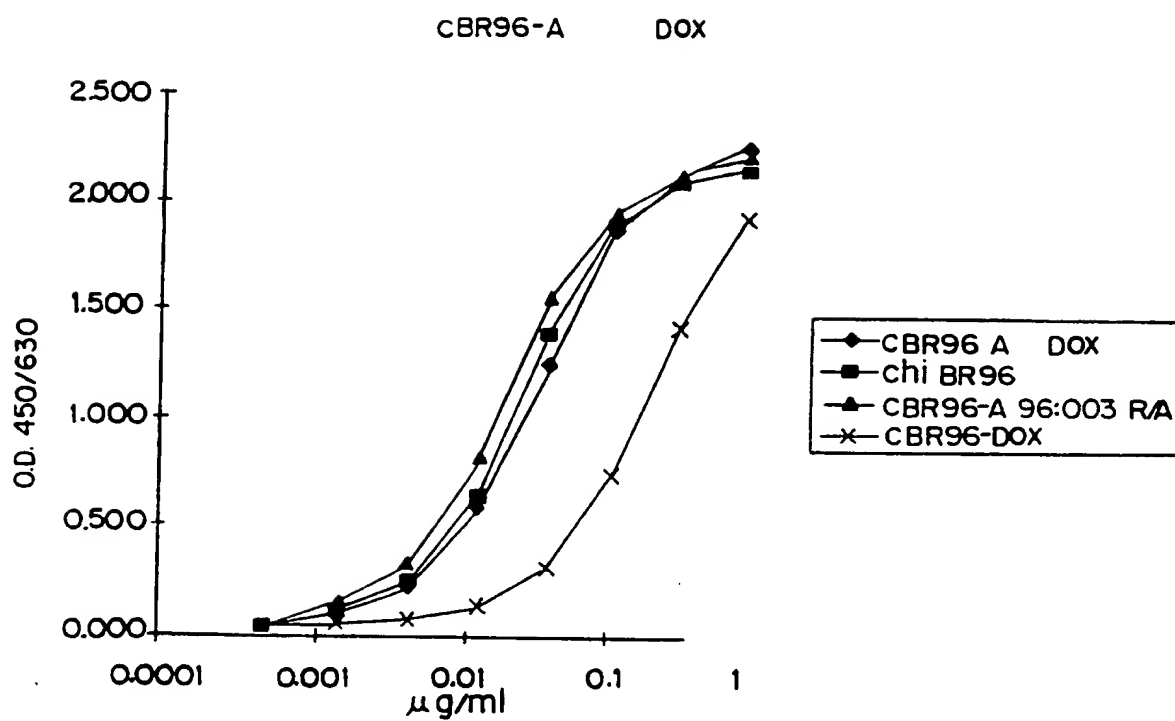
Fig. 5

Fig. 6

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Fig. 7

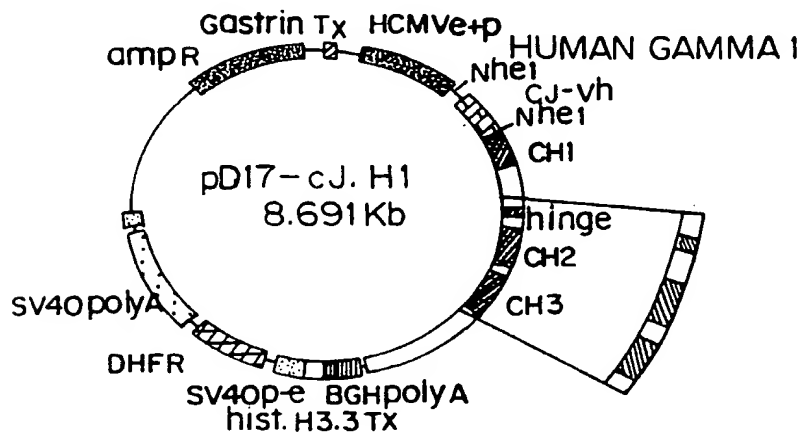
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Fig. 8

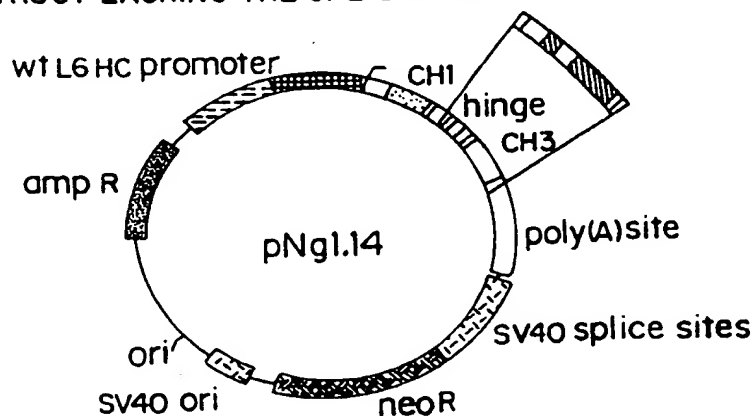
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Fig. 9A

A-HINGE + CH2+CH3 DOMAINS WERE REMOVED FROM BR96 IGG1
CONSTRUCT BY E.CO.47-III RESTRICTION DIGESTION.

**Fig. 9B**

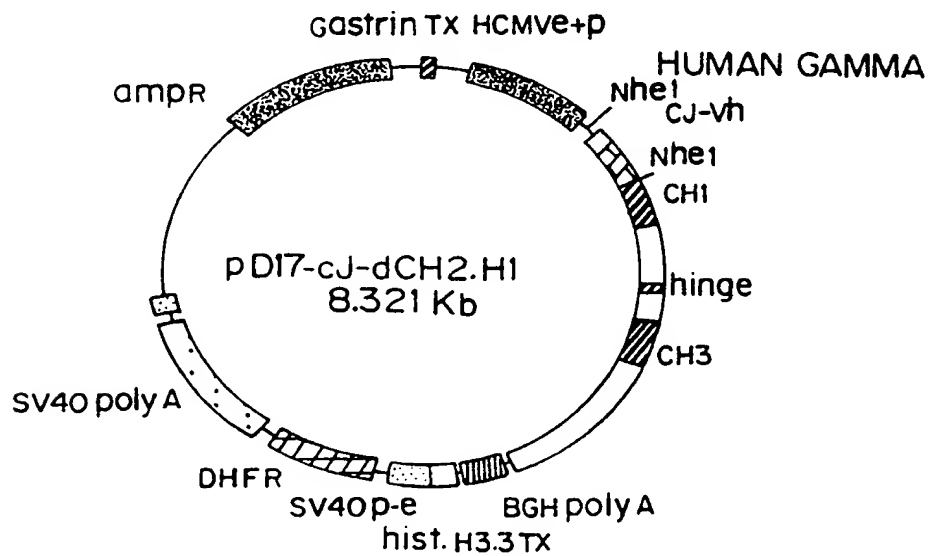
B-HINGE+CH3 DOMAINS AMPLIFIED BY PCR FROM L6 IGG1
CONSTRUCT LACKING THE CH2 DOMAIN.



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Fig. 9C

C-HINGE+CH3 PCR FRAGMENT CLONED BY HOMOLOGOUS
RECOMBINATION INTO E.CO.47-III SITE OF BR96 IGGI MOLECULE.



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1.- INTRODUCTION OF MUTATIONS BY SITE DIRECTED
MUTAGENESIS ON DOUBLE-STRANDED PLASMID DNA.

Fig. 10A

A.- MUTATIONS INTRODUCED INTO SYNTHETIC OLIGONUCLEOTIDES
USED FOR THE PCR AMPLIFICATION OF CH2 DOMAIN

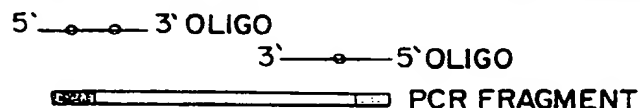


Fig. 10B

B.- PLASMID DNA LINEARIZED INSIDE CH2 DOMAIN AND CO-
TRANSFORMED WITH PCR FRAGMENT INTO COMPETENT DH5 α

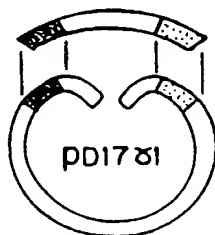
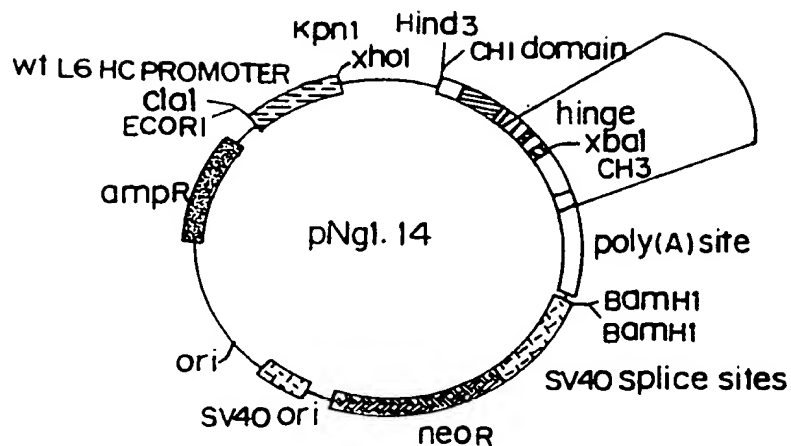
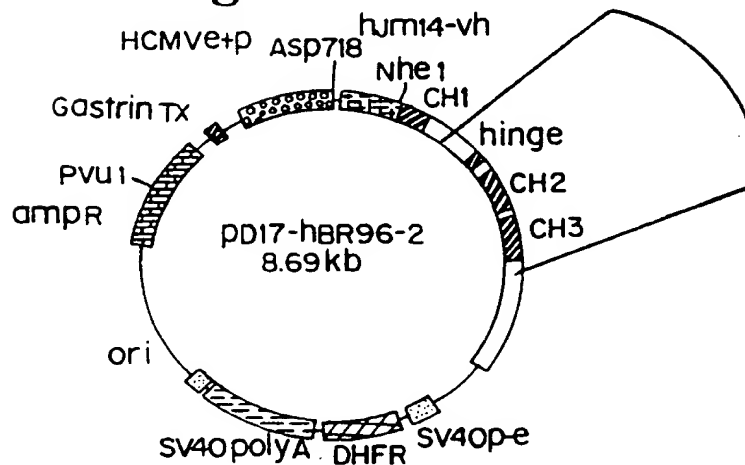
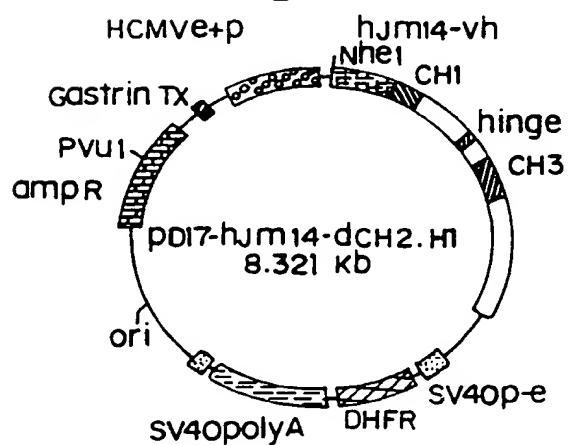


Fig. 10C

C.- CLONING MEDIATED BY HOMOLOGOUS RECOMBINATION YIELDS
TRANSFORMANTS HARBOURING RECOMBINANT PLASMIDS.



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Fig. 11**Fig. 12****Fig. 13**

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FIG. 14A

pd17-cJ-dCH2.H1

10	20	30	40	50	60	70	80	90
GACGGATCGG	GAGATCTGCT	AGGTGACCTG	AGGCGGCGCG	GCTTCGAATA	GCCAGAGTAA	CCTTTTCTTT	TAATTTTATT	TTATTTTATT
CTGCCTAGCC	CTCTAGACGA	TCCACTGGAC	TCCGCGCGCG	CGAAGCTTAT	CGGTCTCATT	GGAATAAAAA	ATTAAAAATA	AATAAAAAATA
100	110	120	130	140	150	160	170	180
TTTGAGATGG	AGTTTGGCGC	CGATCTCCCG	ATCCCTCTATG	GTGCACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC
AAACTCTACC	TCAAACCGCG	GCTAGAGGGC	TAGGGGATAC	CAGCTGAGAG	TCAATGTTAGA	CGAGACTACG	GCGTATCAAT	TCGGTTCATAG
190	200	210	220	230	240	250	260	270
TGCTCCCTGC	TTGTGTGTTG	GAGGTGCGTG	AGTAGTGCGC	GAGCAAAATT	TAAGCTACAA	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA
ACGAGGGACG	AACACACAAC	CTCCAGCGAC	TCATCAGCGC	CTCGTTTAA	ATTTCGATGTT	GTTCCGTTCC	GAACTGGCTG	TTAACGTACT
280	290	300	310	320	330	340	350	360
AGAATCTGCT	TAGGGTTAGG	CGTTTGGCGC	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAAT
TCTTAGACGA	ATCCCAATCC	GCAAAACGCG	ACGAAGCGCT	ACATGCCCGG	TCTATATGCG	CAACTGTAAC	TAATAACTGA	TCAATAATTA
370	380	390	400	410	420	430	440	450
AGTAATCAAT	TACGGGGTCA	TTAGTTTCATA	GCCCATATAT	GGAGTTCGCG	GTTACATAAC	TTACGGTAA	TGGCCCGCCT	GGGTGACCGC
TCATTAGTTA	ATGCCCCAGT	AATCAAGTAT	CGGGTATATA	CCTCAAGGCG	CAATGTATTG	AATGCCATTT	ACGGGGCGGA	CCGACTGGCG
460	470	480	490	500	510	520	530	540
CCAAAGACCC	CCGCCCCATTG	ACGTCAATAA	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT
GGTTGCTGGG	GGCGGGTAAC	TGCAGTTATT	ACTGCATACA	AGGGTATCAT	TGCGGTTATC	CCTGAAAGGT	AACTGCAGTT	ACCCACCTGA
550	560	570	580	590	600	610	620	630
ATTACGGTA	AACTGCCCCAC	TTGGCAGTAC	ATCAAGTGTG	TCATATGCCA	AGTACGCCCC	CTATTGACGT	CAATGACGGT	AAATGGCCCCG
TAAATGCCAT	TTGACGGGTG	AACCGTCATG	TAGTTCACAT	AGTATACGGT	TCATGCGGGG	GATAACTGCA	GTTACTGCCA	TTTACCGGGC
640	650	660	670	680	690	700	710	720
CCTGGCATTA	TGCCCAGTAC	ATGACCTTAT	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGTATGC
GGACCGTAAT	ACGGGTCATG	TACTGGAATA	CCCTGAAAGG	ATGAACCGTC	ATGTAGATGC	ATAATCAGTA	GCGATAATGG	TACCACTACG
730	740	750	760	770	780	790	800	810
GGTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	TCCACCCCCAT	TGACGTCAT	GGGAGTTTGT
CCAAAACCGT	CATGTAGTTA	CCCGACCTTA	TCGCCAARACT	GAGTGCCCTT	AAAGGTTTCA	AGGTGGGTA	ACTGCAGTTA	CCCTCAAACA
820	830	840	850	860	870	880	890	900
TTTGGCACCA	AAATCAACGG	GACTTTCCAA	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGT	CGGTGGGAGG
AAACCGTGGT	TTTAGTTGCC	CTGAAAGGTT	TTACAGCATT	GTTGAGGCGG	GGTAACTGCG	TTTACCCGCC	ATCCGCACAT	GCCACCCCTCC

FIG. 14B

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910	TCTATATAAG	920	CAGAGCTCTC	930	TGGCTAACTA	940	GAGAACCCAC	950	TGCTTACTGG	960	CTTATCGAAA	970	TTAATACGAC	980	TCACTATAGG	990	GAGACCCAAAG
	AGATATATTC		GTCTCGAGAG		ACCGATTGAT		CTCTTGGGTG		ACGAATGACC		GAATAGCTTT		AATTATGCTG		AGTGATATCC		CTCTGGGTTTC
1000		1010	ATTTAAATTG	1020	ATATCTCCTT	1030	AGGTCTCGAG	1040	TCTCTAGATA	1050	ACCGGTCAAT	1060	CGATTGGAAT	1070	TCTTGGGGCC	1080	GCTTGTCTAGC
	GAACCATGGT		TAAATTTAAC		TATAGAGGAA		TCCAGAGCTC		AGAGATCTAT		TGGCCAGTTA		GCTAAACCTTA		AGAACGCCCG		CGAACGATCG
1090		1100	TTGTGGTTAA	1110	GCTTGGTCCT	1120	TCCTTGTCCCT	1130	TGTTTTAAAA	1140	GGTGTCAGT	1150	GTGAAGTGAA	1160	TCTGGTGGAG	1170	TCTGGGGGAG
	CACCATGGAG		AACACCAATT		CGAACCCAGGA		AGGAACAGGA		ACAAAATTTT		CCACAGGTCA		CACCTTCACTT		AGACCCCTC		AGACCCCTC
1180		1190	GCCTGGAGGG	1200	TCCCTGAAAG	1210	TCTCCTGTGT	1220	AACCTCTGGA	1230	TTCACCTTCA	1240	GTGACTATTA	1250	CATGTATTGG	1260	GTTCGCCAGA
	GCTTAGTGCA		CGGACCTCCC		AGGGACTTTC		AGAGGACACA		TTGGAGACCT		AAGTGAAGT		CACCTGATAAT		GTACATAACC		CAAGCGGTCT
1270		1280	GAGGTGGAG	1290	TGGGTGCGAT	1300	ACATTAGTCA	1310	AGGTGGTGAT	1320	ATAACCGACT	1330	ATCCAGACAC	1340	TGTAAAGGGT	1350	CGATTACCCA
	CTCCAGAGAA		CTCCGACCTC		ACCCAGCGTA		TGTAATCAGT		TCCACCACTA		TATTGGCTGA		TAGGTCTGTG		ACATTTCCCA		GCTAAGTGGT
1360		1370	CAATGCCAAG	1380	AACACCCCTGT	1390	ACCTGCAAAAT	1400	GAGCCGTCTG	1410	AAGTCTGAGG	1420	ACACAGCCAT	1430	GTATTACTGT	1440	GCAAGAGGCC
	TCTCCAGAGA		GTTACGGTTC		TTGTGGGACA		TGGACGTTTA		CTCGGCAGAC		TTCAGACTCC		TGTGTCTGTA		CATAATGACA		CGTTCTCCGG
1450		1460	GGCCTGGTTT	1470	GCTTACTGGG	1480	GCCAAGGGAC	1490	TCTGGTCACG	1500	GTCTCTGTAG	1510	CTAGCACCAA	1520	GGGCCCATCG	1530	GTCTTCCCCC
	TGGACGACGG		CCGGACCAAA		CGAATGACCC		CGGTTCCTCTG		AGACCAGTGC		CAGAGACATC		GATCGTGGTT		CCCCGGTAGC		CAGAAGGGGG
1540		1550	CTCCAAGAGC	1560	ACCTCTGGGG	1570	GCACAGCGGC	1580	CCTGGGCTGC	1590	CTGGTCAAGG	1600	ACTACTTCCC	1610	CGAACCGGTG	1620	ACGGTGTCTG
	ACCGTGGGAG		GAGGTCTCTG		TGGAGACCCC		CGTGTGCGCG		GGACCCGACG		GACCAGTTCC		TGATGAAGGG		GCTTGGCCAC		TGCCACAGCA
1630		1640	CGCCCTGACC	1650	AGCGGCGTGC	1660	ACACCTTCCC	1670	GGCTGTCTTA	1680	CAGTCCCTCAG	1690	GACTCTTACTC	1700	CCTCAGCAGC	1710	GTGGTCACCG
	GGAACCTCAGG		CGCGGACTGG		TCGCGGCACG		TGTGGAAGGG		CCGACAGGAT		GTCAGGAGTC		CTGAGATGAG		GGAGTCTGTCG		CACCAGTGGC
1720		1730	TGCGAATGAG	1740	TGGGTCTGGA	1750	GCACCTTGGG	1760	TGTAGACGTT	1770	TTCGGGTCTG	1780	TGTGGTTCCA	1790	CCTGTTCTTT	1800	CAACCACTCT
	ACGGGAGGTC		GTCGAACCCG		TGGGTCTGGA		TGTAGACGTT		TGTAGACGTT		TGTAGACGTT		TGTAGACGTT		TGTAGACGTT		TGTAGACGTT

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FIG. 14C

pD17-cJ-dCH2.H1

1810	1820	1830	1840	1850	1860	1870	1880	1890
GGCCAGCACA	GGGAGGGAGG	GTGTCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGACG	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC
CCGGTCGTGT	CCCTCCCTCC	CACAGACGAC	CTTCGGTCCG	AGTCGCGAGG	ACGACCTGTC	GTAGGGCCGA	TACGTCCGGG	TCAGGTCCCG
1900	1910	1920	1930	1940	1950	1960	1970	1980
AGCAAGGCAG	GCCCCGTCTG	CCTCTTCACC	CGGAGGCCTC	TGCCCCCCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG
TCGTTCCGTC	CGGGGCAGAC	GGAGAAGTGG	GCCTCCGGAG	ACGGGCGGGG	TGAGTACGAG	TCCCTCTCCC	AGAGACCGA	AAAAGGGGTC
1990	2000	2010	2020	2030	2040	2050	2060	2070
GCTCTGGGCA	GGCACAGGCT	AGGTGCCCT	AACCCAGGCC	CTGCACACAA	AGGGGCAGGT	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC
CGAGACCCGT	CCGTGTCCGA	TCCACGGGGA	TTGGGTCCGG	GACGTGTGTT	TCCCCGTCCA	CGACCCGAGT	CTGGACGGTT	CTCGGTATAG
2080	2090	2100	2110	2120	2130	2140	2150	2160
CGGGAGGACC	CTGCCCCCTGA	CCTAAGCCCC	CCCCAAAGGC	CAAACTCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA
GCCCTCCTGG	GACGGGGACT	GGATTCCGGT	GGGGTTTCCG	GTTTGAGAGG	TGAGGGAGTC	GAGCCTGTGG	AAGAGAGGAG	GGTCTAAGGT
2170	2180	2190	2200	2210	2220	2230	2240	2250
GTAACCTCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTC	ACAAACTCA	CACATGCCCA	CCGTGCCCCAG	GTAAGCCAGC	CCAGGCCCTCG
CATTGAGGGT	TAGAAGAGAG	ACGTCTCGGG	TTTAGAACAC	TGTTTGAGT	GTGTACGGGT	GGCACGGGTC	CATTCCGTCTG	GGTCCGGAGC
2260	2270	2280	2290	2300	2310	2320	2330	2340
CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	AGAGTAGCCT	GCATCCAGGG	ACACACCACG	TGGGTACCAA	CATGTCCGGA	GCCACATGGA
GGGAGGTCGA	GTTCGGCCCT	GTCCACGGGA	TCTCATCGGA	CGTAGGTCCC	TGTGTGGTGC	ACCCATGGTT	GTACAGGCCCT	CGGTGTACCT
2350	2360	2370	2380	2390	2400	2410	2420	2430
CAGAGGCCGG	CTCGGCCCCAC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	CCTCTGTCCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA
GTCTCCGGCC	GAGCCGGGTG	GGAGACGGGA	CTCTCACTGG	CGACATGGTT	GGAGACAGGG	ATGTCCCGTC	GGGGCTCTTG	GTGTCCACAT
2440	2450	2460	2470	2480	2490	2500	2510	2520
CACCCTGCCC	CCATCCCGGG	ATGAGCTGAC	CAAGAACCAG	GTCAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCAGCG	ACATCGCCGT
GTGGGACGGG	GGTAGGGCCC	TACTCGACTG	GTTCTTGTC	CAGTCGGACT	GGACGGACCA	GTTTCCGAAG	ATAGGGTCGC	TGTAGCGGCA
2530	2540	2550	2560	2570	2580	2590	2600	2610
GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCCTC	CCGTGCTGGA	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA
CCTCACCCCTC	TCGTTACCCG	TCGGCCTCTT	GTTGATGTTT	TGGTGCGGAG	GGCAGCACCT	GAGGCTGCCG	AGGAAGAAGG	AGATGTCGTT
2620	2630	2640	2650	2660	2670	2680	2690	2700
GCTCACCGTG	GACAAGAGCA	GGTGGCAGCA	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	ACACGCAGAA
CGAGTGGCAC	CTGTTCTCGT	CCACCGTCGT	CCCCTTGCAG	AAGAGTACGA	GGCACTACGT	ACTCCGAGAC	GTGTTGGTGA	TGTGCGTCTT

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FIG. 14D

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2710	2720	2730	2740	2750	2760	2770	2780	2790
GAGCCTCTCC	CTGTCTCCGG	GTAAATGAGT	GGAGGGCCG	GCAAGCCCC	GCTCCCCGG	CTCTCGCGGT	CGACACAGGA	TGCTTGGCAC
CTCGGAGAGG	GACAGAGGCC	CATTTACTCA	CGCTGCCGGC	CGTTCCGGGG	CGAGGGGCCC	GAGAGCGCCA	GCGTGTCTCT	ACGAACCGTG
2800	2810	2820	2830	2840	2850	2860	2870	2880
GTACCCCTCG	TACATACTTC	CCGGGCGCCC	AGCATGGAAA	TAAAGCACCC	AGCGCTGCCC	TGGGCCCCCTG	CGAGACTGTG	ATGGTTCTTT
CATGGGGGAC	ATGTATGAAG	GGCCCGCGGG	TCGTACCTTT	ATTTCTGTGG	TCGCGACGGG	ACCCGGGGAC	GCTCTGACAC	TACCAAGAAA
2890	2900	2910	2920	2930	2940	2950	2960	2970
CCACGGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	GGGTCCCACT	GTCCCCACAC	TGGCCCCAGGC	TGTGCAGGTG
GGTGCCCACT	CCGGCTCAGA	CTCCGGACTC	ACCGTACTCC	CTCCGTCTCG	CCCAGGGTGA	CAGGGGTGTG	ACCGGGTCCG	ACACGTCCAC
2980	2990	3000	3010	3020	3030	3040	3050	3060
TGCTTGGGCC	CCCTAGGGTG	GGGCTCAGCC	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	AGCAGCACCT
ACGGACCCCG	GGGATCCAC	CCCGAGTCGG	TCCCGACCGG	GAGCCGTCCC	ACCCCTAAA	CGTCTGCACC	GGGAGGGAGG	TCGTCTGTGA
3070	3080	3090	3100	3110	3120	3130	3140	3150
GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCCCTGG	GACAGACACA	CAGCCCCCTGC	CTCTGTAGGA	GACTGTCTCTG	TTCTGTGAGC
CGGGACCCGA	CCCGGTGCCC	TTCGGGATCC	TCCGGGACCC	CTGTCTGTGT	GTCCGGGACG	GAGACATCCT	CTGACAGGAC	AAGACACTCG
3160	3170	3180	3190	3200	3210	3220	3230	3240
GCCCCGTGCC	TCCCGACCTC	CATGCCCACT	CGGGGGCATG	CCTAGTCCAT	GTGCGTAGGG	ACAGGCCCTC	CCTCACCCAT	CTACCCCCAC
CGGGACACAG	AGGGCTGGAG	GTACGGGTGA	GCCCCCGTAC	GGATCAGGTA	CACGCATCCC	TGTCCGGGAG	GGAGTGGGTA	GATGGGGGTG
3250	3260	3270	3280	3290	3300	3310	3320	3330
GGCACTAACC	CCTGGCTGCC	CTGCCACGCC	TCGCACCCGC	ATGGGGACAC	AACCGACTCC	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG
CCGTGATTGG	GGACCGACGG	GACGGGTCGG	AGCGTGGCG	TACCCCTGTG	TTGGCTGAGG	CCCCCTGTACG	TGAGAGCCCCG	GGACACCTCC
3340	3350	3360	3370	3380	3390	3400	3410	3420
GACTGGTGCA	GATGCCACCA	CACACACTCA	GCCCAGACCC	GTTCAACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	CACCACACAC
CTGACCACGT	CTACGGGTGT	GTGTGTGAGT	CGGGTCTGGG	CAAGTTGTTT	GGGGCGTGAC	TCCAACCCGGC	CGGTGTGCCG	GTGGTGTGTG
3430	3440	3450	3460	3470	3480	3490	3500	3510
ACACGTGCAC	GCCTCACACA	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	CCCAGACCCAG	AGCAAGGTCC	TGCGACACGT	GAACACTCCT
TGTGCACGTG	CGGAGTGTGT	GCCTCGGAGT	GGGCCCCGCTT	GACGTGTGCT	GGGTCTGGTC	TCGTTCCAGG	AGCGTGTGCA	CTTGTGAGGA
3520	3530	3540	3550	3560	3570	3580	3590	3600
CGGACACAGG	CCCCCAGCAG	CCCCACGCGG	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	TCAGACAAC
GCCTGTGTCC	GGGGTGTCTC	GGGGTGCGCC	GTGGAGTTCC	GGGTCTCGG	AGAGCCGTCG	AAGAGGTGTA	CGACTGGACG	AGTCTGTTTG

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FIG. 14E

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3610	3620	3630	3640	3650	3660	3670	3680	3690
CCAGCCCTCC	TCACACAGG	GTGCCCTGC	AGCCGCCACA	CACACACAGG	GGATCACACA	CCACGTCACG	TCCCTGGCCC	TGGCCCACTT
GGTCGGGAGG	AGAGTGTTC	CACGGGGACG	TCGGCGGTGT	GTGTGTGTCC	CCTAGTGTGT	GGTGCAAGTC	AGGACCGGG	ACCGGGTGAA
3700	3710	3720	3730	3740	3750	3760	3770	3780
CCCAGTGCCG	CCCTTCCCTG	CAGGACGGAT	CAGCCTCGAC	TGTGCCCTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCTTCC	CCCGTGCCCTT
GGGTCACGGC	GGGAAGGGAC	GTCCTGCCCTA	GTCCGGAGCTG	ACACGGGAAG	TCAACGGTCG	GTAGACAACA	AACGGGGAGG	GGGCACGGAA
3790	3800	3810	3820	3830	3840	3850	3860	3870
CCTTGACCCCT	GGAAAGGTGCC	ACTCCCACTG	TCCTTTCCCTA	ATAAATGAG	GAAATTGCAT	CGCATTGTCT	GAGTAGGTGT	CATTCTATTTC
GGAACTGGGA	CC'TCCACGG	TGAGGGGTGAC	AGGAAAGGAT	TATTTTACTC	CTTTAACGTA	CGGTAACAGA	CTCATCCACA	GTAAGATAAG
3880	3890	3900	3910	3920	3930	3940	3950	3960
TGGGGGGTGG	GGTGGGGCAG	GACAGCAAGG	GGGAGGATTG	GGAAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	ATGGCTTCTG
ACCCCCCACC	CCACCCCGTC	CTGTCTGTCC	CCCTCCTAAC	CCTTCTGTTA	TCGTCCGTAC	GACCCCTACG	CCACCCGAGA	TACCGAAGAC
3970	3980	3990	4000	4010	4020	4030	4040	4050
AGGCGGAAAG	AACACAGTGG	GGCTCTAGGG	GGTATCCCCA	CGGCGCCTGT	AGCGGGGCAT	TAAGCGCGGC	GGGTGTGGTG	GTTACGGGCA
TCCGCCTTTC	TTGGTCCACC	CCGAGATCCC	CCATAGGGGT	CGCGGGGACA	TCGCGCGGTA	ATTGCGCGCG	CCCACACCAC	CAATGGCGGT
4060	4070	4080	4090	4100	4110	4120	4130	4140
GGGTGACCGC	TACACTTGCC	AGCGCCCTAG	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCCCT	TTCTGCGCCAC	GTTGCGCGGG	CCTCTCAAAA
CGCACTGGCG	ATGTGAACGG	TCGCGGGATC	GCGGGCGAGG	AAAGCGAAAG	AAGGGAAGGA	AAGAGCGGTG	CAAGCGGCCC	GGAGAGTTTT
4150	4160	4170	4180	4190	4200	4210	4220	4230
AAGGGAANA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	CTAACTCCGC	CCATCCCGCC	CCTAACTCCG	CCCAGTTCCG
TTCCCTTTTT	TTCTGTACGTA	GAGTTAATCA	GTCGTTGGTA	TCAGGGCGGG	GATTGAGGCG	GGTAGGGCGG	GGATTGAGGC	GGGTCAAGGC
4240	4250	4260	4270	4280	4290	4300	4310	4320
CCCATTCTCC	GCCCCATGGC	TGACTAATTT	TTTTTATTTA	TGCAGAGGCC	GAGCGCGCCT	CGGCCTCTGA	GCTATTCCAG	AAGTAGTGAG
GGGTAAGAGG	CGGGGTACCG	ACTGATTAAA	AAAAATAAAT	ACGTCTCCGG	CTCCGGCGGA	GCCGGAGACT	CGATAAGGTC	TTCATCACTC
4330	4340	4350	4360	4370	4380	4390	4400	4410
GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	GCTGCGATTT	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG
CTCCGAAAAA	ACCTCCGGAT	CCGAAAACGT	TTTTTCGAAC	TGTCGAGTCC	CGACGCTAAA	GCGCGGTTTG	AACTGCCGTT	AGGATCGCAC
4420	4430	4440	4450	4460	4470	4480	4490	4500
AAGGCTGGTA	GGATTTTATC	CCCGCTGCCA	TCATGGTTCC	ACCATGTAAC	TGCATCGTCG	CCGTGTCCCA	AAATATGGGG	ATTGGCAAGA
TTCCGACCAT	CCTAAAAATAG	GGGCGACGGT	AGTACCAAGC	TGGTAACCTG	ACGTAGCAGC	GGCACAGGGT	TTTATACCCC	TAACCGTTCT

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FIG. 14F

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4510	ACGGAGACCT	4520	ACCCCTGGCCT	4530	CCGCTCAGGA	4540	ACGAGTTCAA	4550	GTACTTCCAA	4560	AGAATGACCA	4570	CAACCTCTTC	4580	AGTGAAGGT	4590	AAACAGAATC
	TGCCTCTGGA		TGGGACCGGA		GGCGAGTCCT		TGCTCAAGTT		CATGAAGGTT		TCTTACTGGT		GTTGGAGAAG		TCACCTTCCA		TTTGCTCTAG
4600	TGGTGATTAT	4610	GGGTAGGAA	4620	ACCTGGTTCT	4630	CCATTCCTGA	4640	GAAGAATCGA	4650	CCTTTAAAGG	4660	ACAGAATTAA	4670	TATAGTTCTC	4680	AGTAGAGAAC
	ACCACTAATA		CCCATCCTTT		TGGACCAAGA		GGTAAGGACT		CCTTCTTAGCT		GGAAATTTCC		TGTCTTAATT		ATATCAAGAG		TCATCTCTTG
4690	TCAAAGAAC	4700	ACCACGAGGA	4710	GCTCATTTTC	4720	TTGCCAAAAG	4730	TTTGGATGAT	4740	GCCTTAAGAC	4750	TTATTGAACA	4760	ACCGGAATTG	4770	GCAAGTAAAG
	AGTTTCTTGG		TGCTGCTCCT		CGAGTAAAG		AACGGTTTTC		AAACCTACTA		CGGAATTTCTG		ATAAATTTGT		TGGCCTTAAC		CGTTCAATTC
4780	TAGACATGGT	4790	TTGGATAGTC	4800	GGAGGCAGTT	4810	CTGTTTACCA	4820	GGAAGCCATG	4830	AATCAACCAG	4840	GCCACCTTAG	4850	ACTCTTTGTG	4860	ACAAGGATCA
	ATCTGTACCA		AACCTATCAG		CCTCCGTCAA		GACAAATGGT		CCTTCGGTAC		TTAGTTGGTC		CGGTGGAATC		TGAGAAACAC		TGTTCTCTAGT
4870	TGCAGGAATT	4880	TGAAAGTGAC	4890	ACGTTTTTCC	4900	CAGAAATTGA	4910	TTTGGGGAA	4920	TATAAACTTC	4930	TCCCAGAATA	4940	CCCAGGCGTC	4950	CTCTCTGAGG
	ACGTCCTTAA		ACTTTCACTG		TGCAAAAAGG		GTCTTTAACT		AAACCCCTTT		ATATTTGAAG		AGGCTCTTAT		GGGTCCGCAG		GAGAGACTCC
4960	TCCAGGAGGA	4970	AAAAGGCATC	4980	AAGTATAAGT	4990	TTGAAGTCTA	5000	CGAGAAGAA	5010	GACTAACACG	5020	AAGATGCTTT	5030	CAAGTTCTCT	5040	GCTCCCTCTC
	AGGTCCTCCT		TTTTCCGTAG		TTCATATTCA		AACCTCAGAT		GCTCTTCTTT		CTGATTGTCC		TTCTACGAAA		GTTCAAGAGA		CGAGGGGAGG
5050	TAAAGCTATG	5060	CATTTTATATA	5070	AGACCATGGG	5080	ACTTTTGCTG	5090	GCTTTAGATC	5100	TCTTTGTGAA	5110	GGAACCTTAC	5120	TTCTGTGGTG	5130	TGACATAATT
	ATTTCGATAC		GTAAAAATAT		TCTGGTACCC		TGAAAAACGAC		CGAAATCTAG		AGAAACACTT		CCTTGAATG		AAGACACCAC		ACTGTATTAA
5140	GGACAACTA	5150	CCTACAGAGA	5160	TTTAAAGCTC	5170	TAAGGTAAAT	5180	ATAAAATTTT	5190	TAAGTGTATA	5200	ATGTGTTAAA	5210	CTACTGATTC	5220	TAATGTTTGG
	CCTGTTTGAT		GGATGTCTCT		AAATTTGAG		ATTCCATTTA		TATTTTAAAA		ATTCACATAT		TACACAATTT		GATGACTAAG		ATTAACAAAC
5230	TGTATTTTAG	5240	ATTCCAACCT	5250	ATGGAACCTGA	5260	TGAATGGGAG	5270	CAGTGGTGGA	5280	ATGCCTTTAA	5290	TGAGGAAAAC	5300	CTGTTTTGCT	5310	CAGAAGAAAT
	ACATAAAATC		TAAGGTTGGA		TACCTTGACT		ACTTACCCTC		GTCACCACCT		TACGGAATTT		ACTCCTTTTG		GACAAAACGA		GTCTTCTTTA
5320	GCCATCTAGT	5330	GATGATGAGG	5340	CTACTGCTGA	5350	CTCTCAACAT	5360	TCTACTCCTC	5370	CAAAAAAGAA	5380	GAGAAAGGTA	5390	GAAAGCCCCA	5400	AGGACTTTCC
	CGGTAGATCA		CTACTACTCC		GATGACGACT		GAGAGTTGTA		AGATGAGGAG		GTTTTTTCTT		CTCTTTCCAT		CTTCTGGGGT		TCCTGAAAGG

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FIG. 14G

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5410	5420	5430	5440	5450	5460	5470	5480	5490
TTCAGAAATG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTAGT	AATAGAATC	TTGCTTGCTT	TGCTATTATC	ACCACAAAGG	AAAAAGCTGC
AAGTCTTAAC	GATTCAAAA	ACTCAGTACG	ACACAAATCA	TTATCTTGAG	AACGAACGAA	ACGATAAATG	TGGTGTTTCC	TTTTTCGACG
5500	5510	5520	5530	5540	5550	5560	5570	5580
ACTGCTATAC	AAGAAAATTA	TGGAAAATA	TTCTGTAACC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	TTTTTCTTAC
TGACGATATG	TTCTTTTAAT	ACCTTTTAT	AAGACATTGG	AAATATTAT	CCGTATTGTC	AATATTAGTA	TTGTATGACA	AAAAAGAATG
5590	5600	5610	5620	5630	5640	5650	5660	5670
TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	CAAAAATTGT	GTACCTTTAG	CTTTTAAAT	TGTAAAGGGG	TTAATAAGGA
AGGTGTGTCC	GTATCTCACA	GACGATAATT	ATTGATACGA	GTTTTTAACA	CATGGAATC	GAAAAATTAA	ACATTTCCCC	AATTATTCCCT
5680	5690	5700	5710	5720	5730	5740	5750	5760
ATATTTGATG	TATAGTGCCT	TGACTAGAGA	TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTTTACTTG	TTTAAAAAAC	CTCCCCACACC
TATAAACTAC	ATATCACGGA	ACTGATCTCT	AGTATTAGTC	GGTATGGTGT	AAACATCTCC	AAAAATGAACG	AAATTTTGTG	GAGGGTGTGG
5770	5780	5790	5800	5810	5820	5830	5840	5850
TCCCCCTGAA	CCTGAAACAT	AAATGAATG	CAATTGTTGT	TGTTAACTTG	TTTATTGCAG	CTTATAATGG	TTACAAATAA	AGCAATAGCA
AGGGGGACTT	GGACTTTGTA	TTTTACTTAC	GTTAACACAA	ACAATTGAAC	AAATAACGTC	GAATATTACC	AATGTTTATT	TCGTTATCGT
5860	5870	5880	5890	5900	5910	5920	5930	5940
TCACAAATTT	CACAAATAAA	GCATTTTTTT	CACTGCATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	GTCTGGATCG
AGTGTTTAAA	GTGTTTATTT	CGTAAAAAAA	GTGACGTAAG	ATCAACACCA	AACAGGTTTG	AGTAGTTACA	TAGAATAGTA	CAGACCTAGC
5950	5960	5970	5980	5990	6000	6010	6020	6030
GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCCAC	CCCAACTTGT	TTATTGCAGC	TTATAATGGT	TACAAATAAA
CGACCTACTA	GGAGGTCGCG	CCCCTAGAGT	ACGACCTCAA	GAAGCGGGTG	GGGTTGAACA	AATAACGTCG	AATATTACCA	ATGTTTATTT
6040	6050	6060	6070	6080	6090	6100	6110	6120
GCAATAGCAT	CACAAATTTT	ACAAATAAAG	CATTTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	TCATTATCATG
CGTTATCGTA	GTGTTTAAAG	TGTTTATTTT	GTAAAAAAG	TGACGTAAGA	TCAACACCAA	ACAGGTTTGA	GTAGTTACAT	AGAAATAGTAC
6130	6140	6150	6160	6170	6180	6190	6200	6210
TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCTCTG	TGTGAATTTG	TTATCCGCTC	ACAATTCAC
AGACATATGG	CAGCTGGAGA	TCGATCTCGA	ACCGCATTTAG	TACCAGTATC	GACAAAGGAC	ACACTTTAAC	AATAGGCGAG	TGTTAAGGTG
6220	6230	6240	6250	6260	6270	6280	6290	6300
ACAACATACG	AGCCGGAAGC	ATAAAGTGTA	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAAT	TGCGTTGCGC	TCACTGCCCC
TGTTGTATGC	TCGGCCTTCG	TATTTTACAT	TTCCGACCCC	ACGGATTACT	CACTCGATTG	AGTGTAAATTA	ACGCAACGCG	AGTGACGGGC

FIG. 14H

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6310	6320	6330	6340	6350	6360	6370	6380	6390
CTTTCCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAAATG	AATCGGCCAA	CGCGCGGGA	GAGCGGTTT	GGTATTGGG	CGCTCTTCCG
GAAAGGTCAG	CCCTTTGGAC	AGCACGGTCG	ACGTAATTAC	TTAGCCGGTT	CGCGGCCCTT	CTCCGCCAAA	CGCATAACCC	CGGAGAAGGC
6400	6410	6420	6430	6440	6450	6460	6470	6480
CTTCCTCGCT	CACTGACTCG	CTGCGCTCGG	TCGTTCCGCT	GCGCGGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG
GAAAGAGCGA	GTGACTGAGC	GACGCGAGCC	AGCAAGCCGA	CGCCGCTCGC	CATAGTCGAG	TGAGTTTCCG	CCATTATGCC	AATAGGTGTC
6490	6500	6510	6520	6530	6540	6550	6560	6570
AATCAGGGGA	TAACGCAGGA	AAGAATCATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCC
TTAGTCCCTT	ATTGCGTCCT	TTCTTGATACA	CTCGTTTTCC	GGTCGTTTTT	CGTCCCTTGG	CATTTTTTCCG	GCGCAACGAC	CGCAAAAAGG
6580	6590	6600	6610	6620	6630	6640	6650	6660
ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAATCCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT
TATCCGAGGC	GGGGGACTG	CTCGTAGTGT	TTTTAGCTGC	GAGTTCACTC	TCCACCGCTT	TGGGCTGTCC	TGATATTTCT	ATGGTCCGCA
6670	6680	6690	6700	6710	6720	6730	6740	6750
TTCCCCCTGG	AAGTCCCTC	GTGCGCTCTC	CTGTTCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCCCT	TCTCCCTTCG	GGAAGCGTGG
AAGGGGACC	TTCGAGGGAG	CACGCGAGAG	GACAAAGGCTG	GGACGGCGAA	TGGCCTATGG	ACAGGGCGAA	AGAGGGAAGC	CCTTCGCACC
6760	6770	6780	6790	6800	6810	6820	6830	6840
CGCTTTCTCA	ATGCTCACGC	TGTAGGTATC	TCAGTTCCGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTTCAGC
GCGAAAGAGT	TACGAGTGCG	ACATCCATAG	AGTCAAGCCA	CATCCAGCAA	GCGAGGTTCC	ACCCGACACA	CGTGTCTGGG	GGGCAAGTCG
6850	6860	6870	6880	6890	6900	6910	6920	6930
CCGACCGCTG	CGCCTTATCC	GGTAACATATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATGCCCACT	GGCAGCAGCC	ACTGGTAACA
GGCTGGCGAC	GCGGAATAGG	CCATTGATAG	CAGAACTCAG	GTTGGGCCAT	TCTGTGCTGA	ATAGCGGTGA	CCGTGCTCGG	TGACCATTTGT
6940	6950	6960	6970	6980	6990	7000	7010	7020
GGATTAGCAG	AGCGAGGAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTTGGTA
CCTAATCGTC	TCGCTCCATA	CATCCGCCAC	GATGTCTCAA	GAACTTCACC	ACCGGATTGA	TGCCGATGTG	ATCTTCTCTG	CATAAACCAT
7030	7040	7050	7060	7070	7080	7090	7100	7110
TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCCAC	CGCTGGTAGC	GGTGGTTTTT
AGACGGGAGA	CGACTTCGGT	CAATGGGAAGC	CTTTTTCTCA	ACCATCGAGA	ACTAGGCCGT	TTGTTTGGTG	GCGACCATCG	CCACCACAAA
7120	7130	7140	7150	7160	7170	7180	7190	7200
TTGTTTGCAA	GCAGCAGATT	ACGGCGCAGAA	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAAACG
AACAAACGTT	CGTCGTCTAA	TGCGCGTCTT	TTTTTCTCTAG	AGTTCTTCTA	GGAACTAGA	AAAGATGCCC	CAGACTGCGA	GTACACCTTGC

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FIG. 14I

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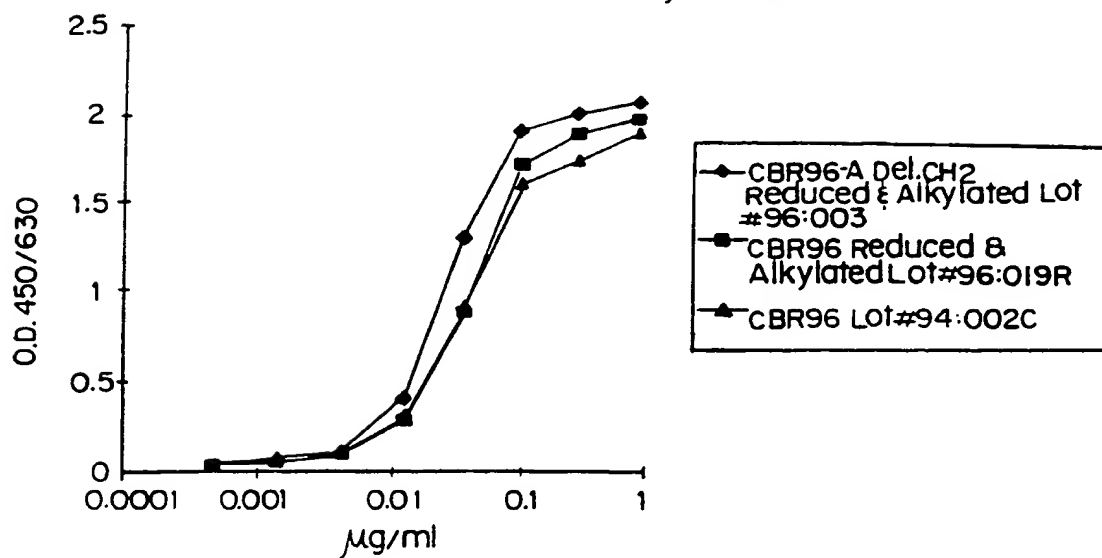
7210	7220	7230	7240	7250	7260	7270	7280	7290
AAACTCAG	TTAAGGATT	TTGGTCATGA	GATTATCAA	AAGGATCTTC	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA
TTTTGAGTGC	AATTCCTAA	AACCACTACT	CTAATAGTTT	TTCTAGAAG	TGGATCTAGG	AAAATTTAAT	TTTTACTTCA	AAATTTAGTT
7300	7310	7320	7330	7340	7350	7360	7370	7380
TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTCAT
AGATTTCATA	TATACTCATT	TGAACCAAGAC	TGTCAAATGGT	TACGAATTAG	TCACTCCGTG	GATAGAGTCG	CTAGACAGAT	AAAGCAAGTA
7390	7400	7410	7420	7430	7440	7450	7460	7470
CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT	ACGGGAGGGC	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	CCGCGAGAGCC
GGTATCAACG	GACTGAGGGG	CAGCACATCT	ATTGATGCTA	TGCCCTCCCG	AATGGTAGAC	CGGGGTACAG	ACGTTACTAT	GGCGCTCTGG
7480	7490	7500	7510	7520	7530	7540	7550	7560
CACGCTCACC	GGCTCCAGAT	TTATCAGCAA	TAAACCAAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAAGTGTCC	TGCAACTTTA	TCCGCCCTCCA
GTGCGAGTGG	CCGAGGTCTA	AATAGTCGTT	ATTGGTCCG	TCCGCCCTCC	CGGCTCGCGT	CTTCACCAGG	ACGTTGAAAT	AGGCGGAGGT
7570	7580	7590	7600	7610	7620	7630	7640	7650
TCCAGTCTAT	TAATGTGTGC	CGGGAAGCTA	GAGTAAGTAG	TTCGCCAGTT	AATAGTTTGC	GCAACGTTGT	TGCCATTTGCT	ACAGGCATCG
AGGTCAGATA	ATTAACAACG	GCCCTTCGAT	CTCATTCATC	AAGCGGTCAA	TTATCAAACG	CGTTGCAACA	ACGGTAACGA	TGTCGGTAGC
7660	7670	7680	7690	7700	7710	7720	7730	7740
TGGTGTACAG	CTCGTCGTTT	GGTATGGCTT	CATTCAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGTGCAAAA
ACCACAGTGC	GAGCAGCAAA	CCATACCCGAA	GTAAGTCGAG	GCCAAAGGTT	GCTAGTTCCG	CTCAATGTAC	TAGGGGGTAC	AACACGTTTT
7750	7760	7770	7780	7790	7800	7810	7820	7830
AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAAGTTGSCC	GCAGTGTAT	CACTCATGGT	TATGGCAGCA	CTGCATAATT
TTCCGCAATC	GAGGAAGCCA	GGAGGCTAGC	AACAGTCTTC	ATTCAAACCGG	CGTCACAATA	GTGAGTACCA	ATACCGTCGT	GACGTATTAA
7840	7850	7860	7870	7880	7890	7900	7910	7920
CTCTTACTGT	CATGCCATCC	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGGGACCGA
GAGAATGACA	GTACGGTAGG	CATTCTACGA	AAAGACACTG	ACCACCTCATG	AGTTGGTTCA	GTAAGACTCT	TATCACATAC	GCGGCTGGCT
7930	7940	7950	7960	7970	7980	7990	8000	8010
GTTGTCTTTG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAG	TGCTCATCAT	TGGAACACGT	TCCTCGGGGC
CAACGAGAAC	GGGCGGCAGT	TATGCCCTAT	TATGGGCGCG	TGTATCGTCT	TGAAATTTTC	ACGAGTAGTA	ACCTTTTGCA	AGAAGCCCCG
8020	8030	8040	8050	8060	8070	8080	8090	8100
GAAACTCTC	AAGGATCTTA	CCGCTGTTGA	GATCCAGTTC	GATGTAACCC	ACTCGTGAC	CCAACGTGATC	TTCAGCATCT	TTTACTTTCA
CTTTTGAGAG	TTCTTAGAAT	GGCGACAACT	CTAGGTCAAG	CTACATTTGG	TGAGCACGTG	GGTTGACTAG	AAGTCGTAGA	AAATGAAAGT

FIG. 14J

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8110	8120	8130	8140	8150	8160	8170	8180	8190
CCAGCGTTTC	TGGGTGAGCA	AAACACAGGAA	GGCAAAATGC	CGCAAAAAG	GGATTAAGG	CGACACGGAA	ATGTTGAATA	CTCATACTCT
GGTCGCAAG	ACCCACTCGT	TTTTGTCCCTT	CCGTTTACG	GCGTTTTTC	CCTTATTCCC	GCTGTGCCCTT	TACAACTTAT	GAGTATGAGA
8200	8210	8220	8230	8240	8250	8260	8270	8280
TCCTTTTCA	ATATTATTGA	AGCATTATC	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG
AGGAAAAAGT	TATAATAACT	TCGTAAATAG	TCCCAATAAC	AGAGTACTCG	CCTATGTATA	AACTTACATA	AATCTTTTAA	TTTGTTTATC
8290	8300	8310	8320	8330				
GGGTTCCGCG	CACATTTC	CGAAAAGTGC	CACCTGACGT	C				
CCCAAGGCGC	GTGTAAAGG	GCTTTTCACG	GTGGACTGCA	G				

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*Fig. 15*COMPARISON OF WHOLE chiBR96 AND
DELETED CH2 chiBR96 ON Ley/K ELISA

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hBR96-2B:L235 to A235 and G237 to A237

hBR96-2C:E318 to S318, K320 to S320, and K322 to S322

hBR96-2D:P331 to A331

hBR96-2E:L235 to A235, G237 to A237, E318 to S318, K320 to S320,
and K322 to S322

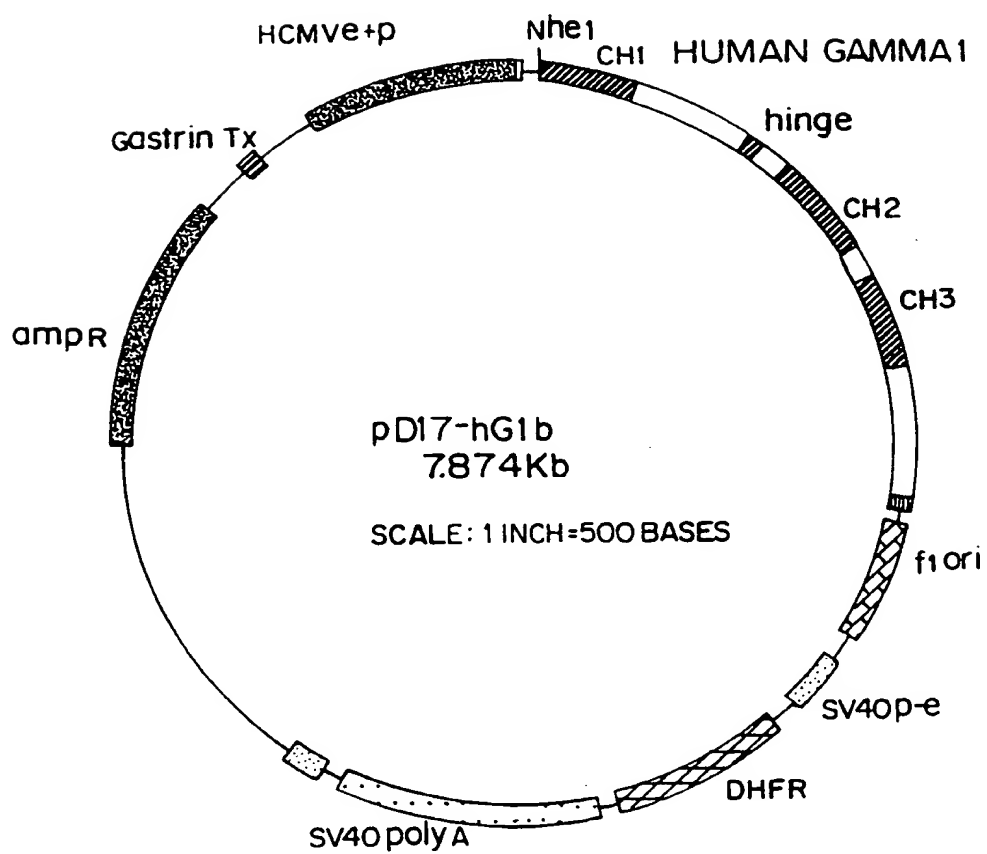
hBR96-2F:L235 to A235, G237 to A237, and P331 to A331

hBR96-2G:E318 to S318, K320 to S320, K322 to S322, and P331 to
A331

hBR96-2H: L235 to A235, G237 to A237, E318 to S318, K320 to S320,
K322 to S322, and P331 to A331

FIG. 16**SUBSTITUTE SHEET (RULE 26)**

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Fig. 17

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FIG. 18A

1	GGTACCAATT	TAAATTGATA	TCTCCTTAGG	TCTCGAGTCT	CTAGATAACC
51	GGTCAATCGA	TTGGAATTCT	TGCGGCCGCT	TGCTAGCCAC	CATGGAGTTG
101	TGGTTAAGCT	TGGTCTTCCT	TGTCCTTGTT	TTAAAAGGTG	TCCAGTGTGA
151	AGTGCAACTG	GTGGAGTCTG	GGGGAGGCTT	AGTGCAGCCT	GGAGGGTCCC
201	TGCGACTTTC	CTGTGCTGCA	TCTGGATTCC	CGTTCAGTGA	CTATTACATG
251	TATTGGGTTC	GCCAGGCTCC	AGGCAAGGGA	CTGGAGTGGG	TCTCATACAT
301	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT
351	TCACCATCTC	CAGAGACAAT	GCAAAGAACA	GCCTGTACCT	GCAAATGAAC
401	AGCCTGAGGG	ACGAGGACAC	AGCCGTGTAT	TACTGTGCAA	GAGGCCTGGC
451	GGACGGGGCC	TGGTTTGCTT	ACTGGGGCCA	AGGGACTCTG	GTCACGGTCT
501	CTTCCGCTAG	CACCAAGGGC	CCATCGGTCT	TCCCCCTGGC	ACCCTCCTCC
551	AAGAGCACCT	CTGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACTA
601	CTTCCCCGAA	CCGGTGACGG	TGTCGTGGAA	CTCAGGCGCC	CTGACCAGCG
651	GCGTGACAC	CTTCCCGGCT	GTCTACAGT	CCTCAGGACT	CTACTCCCTC
701	AGCAGCGTGG	TCACCGTGCC	CTCCAGCAGC	TTGGGCACCC	AGACCTACAT
751	CTGCAACGTG	AATCACAAGC	CCAGCAACAC	CAAGGTGGAC	AAGAAAGTTG
801	GTGAGAGGCC	AGCACAGGGA	GGGAGGGTGT	CTGCTGGAAG	CCAGGCTCAG
851	CGCTCCTGCC	TGGACGCATC	CCGGCTATGC	AGCCCCAGTC	CAGGGCAGCA
901	AGGCAGGCCC	CGTCTGCCTC	TTCACCCGGA	GGCCTCTGCC	CGCCCCACTC
951	ATGCTCAGGG	AGAGGGTCTT	CTGGCTTTTT	CCCCAGGCTC	TGGGCAGGCA
1001	CAGGCTAGGT	GCCCCTAACC	CAGGCCCTGC	ACACAAAGGG	GCAGGTGCTG
1051	GGCTCAGACC	TGCCAAGAGC	CATATCCGGG	AGGACCCTGC	CCCTGACCTA
1101	AGCCCACCCC	AAAGGCCAAA	CTCTCCACTC	CCTCAGCTCG	GACACCTTCT
1151	CTCCTCCAG	ATTCCAGTAA	CTCCCAATCT	TCTCTCTGCA	GAGCCCAAAT
1201	CTTGTGACAA	AACTCACACA	TGCCCACCGT	GCCCAGGTAA	GCCAGCCCAG
1251	GCCTCGCCCT	CCAGCTCAAG	GCGGGACAGG	TGCCCTAGAG	TAGCCTGCAT
1301	CCAGGGACAG	GCCCCAGCCG	GGTGCTGACA	CGTCCACCTC	CATCTCTTCC

SUBSTITUTE SHEET (RULE 26)

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		235	237		
1351	TCAGCACCTG	AACTCCTGGG	GGGACCGTCA	GTCTTCCTCT	TCCCCCAAA
1401	ACCCAAGGAC	ACCCTCATGA	TCTCCCGGAC	CCCTGAGGTC	ACATGCGTGG
1451	TGGTGGACGT	GAGCCACGAA	GACCCGTGAGG	TCAAGTTCAA	CTGGTACGTG
1501	GACGGCGTGG	AGGTGCATAA	TGCCAAGACA	AAGCCGCGGG	AGGAGCAGTA
1551	CAACAGCACG	TACCGTGTGG	TCAGCGTCCT	CACCGTCCTG	CACCAGGACT
		318	320 322		
1601	GGCTGAATGG	CAAGGAGTAC	AAGTGCAAGG	TCTCCAACAA	AGCCCTCCCA
	331				
1651	GCCCCATCG	AGAAAACCAT	CTCCAAAGCC	AAAGGTGGGA	CCCGTGGGGT
1701	GCGAGGGCCA	CATGGACAGA	GGCCGGCTCG	GCCCACCCTC	TGCCCTGAGA
1751	GTGACCGCTG	TACCAACCTC	TGTCCCTACA	GGGCAGCCCC	GAGAACCACA
1801	GGTGTACACC	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG	AACCAGGTCA
1851	GCCTGACCTG	CCTGGTCAAA	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG
1901	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	TACAAGACCA	CGCCTCCCGT
1951	GCTGGACTCC	GACGGCTCCT	TCTTCCTCTA	CAGCAAGCTC	ACCGTGGACA
2001	AGAGCAGGTG	GCAGCAGGGG	AACGTCTTCT	CATGCTCCGT	GATGCATGAG
2051	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA
2101	ATGAGTGCGA	CGGCCGGCAA	GCCCCGCTC	CCCGGGCTCT	CGCGGTGCGA
2151	CGAGGATGCT	TGGCACGTAC	CCCCTGTACA	TACTTCCCGG	GCGCCCAGCA
2201	TGGAAATAAA	GCACCCAGCG	CTGCCCTGGG	CCCCTGCGAG	ACTGTGATGG
2251	TTCTTTCCAC	GGGTCAGGCC	GAGTCTGAGG	CCTGAGTGGC	ATGAGGGAGG
2301	CAGAGCGGGT	CCCACTGTCC	CCCACTGGC	CCAGGCTGTG	CAGGTGTGCC
2351	TGGGCCCCCT	AGGGTGGGGC	TCAGCCAGGG	GCTGCCCTCG	GCAGGGTGGG
2401	GGATTTGCCA	GCGTGGCCCT	CCCTCCAGCA	GCACCTGCCC	TGGGCTGGGC
2451	CACGGGAAGC	CCTAGGAGCC	CCTGGGGACA	GACACACAGC	CCCTGCCTCT
2501	GTAGGAGACT	GTCCTGTTCT	GTGAGCGCCC	CTGTCCTCCC	GACCTCCATG
2551	CCCACTCGGG	GGCATGCCTA	GTCCATGTGC	GTAGGGACAG	GCCCTCCCTC
2601	ACCCATCTAC	CCCCACGGCA	CTAACCCTTG	GCTGCCCTGC	CCAGCCTCGC
2651	ACCCGCATGG	GGACACAACC	GACTCCGGGG	ACATGCACTC	TCGGGCCCTG
2701	TGGAGGGACT	GGTGCAGATG	CCCACACACA	CACTCAGCCC	AGACCCGTTC
2751	AACAAACCCC	GCACTGAGGT	TGGCCGGCCA	CACGGCCACC	ACACACACAC
2801	GTGCACGCCT	CACACACGGA	GCCTCACCCG	GGCGAACTGC	ACAGCACCCA

FIG. 18B

SUBSTITUTE SHEET (RULE 26)

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2851	GACCAGAGCA	AGGTCCTCGC	ACACGTGAAC	ACTCCTCGGA	CACAGGCCCC
2901	CACGAGCCCC	ACGCGGCACC	TCAAGGCCCA	CGAGCCTCTC	GGCAGCTTCT
2951	CCACATGCTG	ACCTGCTCAG	ATAAACCCAG	CCCTCCTCTC	ACAAGGGTGC
3001	CCCTGCAGCC	GCCACACACA	CACAGGGGAT	CACACACCAC	GTCACGTCCC
3051	TGGCCCTGGC	CCACTTCCCA	GTGCCGCCCT	TCCCTGCAGG	ACGGATCAGC
3101	CTCGACTGTG	CCTTCTAGTT	GCCAGCCATC	TGTTGTTTGC	CCCTCCCCCG
3151	TGCCCTTCCTT	GACCCTGGAA	GGTGCCACTC	CCACTGTCTT	TTCCTAATAA
3201	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT	CTATTCTGGG
3251	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAATAGCA
3301	GGCATGCTGG	GGATGCGGTG	GGTCTATGG	CTTCTGAGGC	GGAAAGAACC
3351	AGCTGGGGCT	CTAGGGGGTA	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAAG
3401	CGCGGCGGGT	GTGGTGGTTA	CGCGCAGCGT	GACCGCTACA	CTTGCCAGCG
3451	CCCTAGCGCC	CGCTCCTTTC	GCTTCTTTC	CTTCCTTCT	CGCCACGTTC
3501	GCCGGGCCTC	TCAAAAAAGG	GAAAAAAGC	ATGCATCTCA	ATTAGTCAGC
3551	AACCATAGTC	CCGCCCCCTAA	CTCCGCCCAT	CCCGCCCCCTA	ACTCCGCCCA
3601	GTTCCGCCCA	TTCTCCGCCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA
3651	GAGGCCGAGG	CCGCCTCGGC	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG
3701	CTTTTTTTGGA	GGCCTAGGCT	TTTGCAAAAA	GCTTGACACAG	CTCAGGGCTG
3751	CGATTTTCGCG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	CTGGTAGGAT
3801	TTTATCCCCG	CTGCCATCAT	GGTTCGACCA	TTGAACTGCA	TCGTCGCCGT
3851	GTCCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC
3901	TCAGGAACGA	GTTCAAGTAC	TTCCAAAGAA	TGACCACAAC	CTCTTCAGTG
3951	GAAGGTAAAC	AGAATCTGGT	GATTATGGGT	AGGAAAACCT	GGTTCTCCAT
4001	TCCTGAGAAG	AATCGACCTT	TAAAGGACAG	AATTAATATA	GTTCTCAGTA
4051	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	ATTTTCTTGC	CAAAAGTTTG
4101	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	GTAAAGTAGA
4151	CATGGTTTGG	ATAGTCGGAG	GCAGTTCGT	TTACCAGGAA	GCCATGAATC
4201	AACCAGGCCA	CCTTAGACTC	TTGTGACAA	GGATCATGCA	GGAAATTTGAA
4251	AGTGACACGT	TTTTCCCAGA	AATTGATTTG	GGGAAATATA	AACCTCTCCC
4301	AGAATACCCA	GGCGTCCTCT	CTGAGGTCCA	GGAGGAAAAA	GGCATCAAGT

FIG. 18C

SUBSTITUTE SHEET (RULE 26)

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4351	ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	TGCTTTCAAG
4401	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT
4451	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC
4501	ATAATTGGAC	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA
4551	AATTTTTTAAG	TGTATAATGT	GTTAAACTAC	TGATTCTAAT	TGTTTGTGTA
4601	TTTGTAGATTC	CAACCTATGG	AACTGATGAA	TGGGAGCAGT	GGTGGAATGC
4651	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	AGAAATGCCA	TCTAGTGATG
4701	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	AAAGAAGAGA
4751	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTTGAG
4801	TCATGCTGTG	TTTAGTAATA	GAACCTTGC	TTGCTTTGCT	ATTTACACCA
4851	CAAAGGAAAA	AGCTGCACTG	CTATACAAGA	AAATTATGGA	AAAATATTCT
4901	GTAACCTTTA	TAAGTAGGCA	TAACAGTTAT	AATCATAACA	TACTGTTTTT
4951	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	TATGCTCAAA
5001	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTAA	TAAGGAATAT
5051	TTGATGTATA	GTGCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTTG
5101	TAGAGGTTTT	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG
5151	AAACATAAAA	TGAATGCAAT	TGTTGTTGTT	AACTTGTTTA	TTGCAGCTTA
5201	TAATGGTTAC	AAATAAAGCA	ATAGCATCAC	AAATTTCACA	AATAAAGCAT
5251	TTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	CCAAACTCAT	CAATGTATCT
5301	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	ATCTCATGCT
5351	GGAGTTCTTC	GCCCACCCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA
5401	AATAAAGCAA	TAGCATCACA	AATTTCACAA	ATAAAGCATT	TTTTTCACTG
5451	CATTCTAGTT	GTGGTTTGTG	CAAACCTCAT	AATGTATCTT	ATCATGTCTG
5501	TATACCGTCG	ACCTCTAGCT	AGAGCTTGGC	GTAATCATGG	TCATAGCTGT
5551	TTCTGTGTG	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	CATACGAGCC
5601	GGAAGCATAA	AGTGTAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC
5651	ATTAATTGCG	TTGCGCTCAC	TGCCCCGCTT	CCAGTCGGGA	AACCTGTCGT
5701	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCCT
5751	ATTGGGCGCT	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	GCTCGGTCGT
5801	TCGGCTGCGG	CGAGCGGTAT	CAGCTCACTC	AAAGGCGGTA	ATACGGTTAT

FIG. 18D

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5851	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	AAAAGGCCAG
5901	CAAAAGGCCA	GGAACCGTAA	AAAGGCCCGC	TTGCTGGCGT	TTTTCCATAG
5951	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT
6001	GGCGAAACCC	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC
6051	TCCCTCGTGC	GCTCTCCTGT	TCCGACCCTG	CCGCTTACCG	GATACCTGTC
6101	CGCCTTTCTC	CCTTCGGGAA	GCGTGCGCT	TTCTCAATGC	TCACGCTGTA
6151	GGTATCTCAG	TTCGGTGTAG	GTCGTTGCT	CCAAGCTGGG	CTGTGTGCAC
6201	GAACCCCCCG	TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT
6251	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG
6301	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCCTG
6351	AAGTGGTGGC	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG
6401	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT
6451	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	TTGCAAGCAG
6501	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC
6551	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTAA	GGGATTTTGG
6601	TCATGAGATT	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAAAAA
6651	TGAAGTTTTA	AATCAATCTA	AAGTATATAT	GAGTAAACTT	GGTCTGACAG
6701	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	CTCAGCGATC	TGTCTATTTT
6751	GTTTCATCCAT	AGTTGCCTGA	CTCCCCGTCG	TGTAGATAAC	TACGATACGG
6801	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA	ATGATACCGC	GAGACCCACG
6851	CTCACC GGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG
6901	AGCGCAGAAG	TGGTCCTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAAT
6951	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCG	CCAGTTAATA	GTTTGCGCAA
7001	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	GTCACGCTCG	TCGTTTGGTA
7051	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	TACATGATCC
7101	CCCATGTTGT	GCAAAAAGC	GGTTAGCTCC	TTCGGTCCTC	CGATCGTTGT
7151	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC
7201	ATAATTCTCT	TACTGTCATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT
7251	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG
7301	CTCTTGCCCC	GCGTCAATAC	GGGATAATAC	CGCGCCACAT	AGCAGAACTT

FIG. 18E

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7351	TAAAAGTGCT	CATCATTGGA	AAACGTTCTT	CGGGGCGAAA	ACTCTCAAGG
7401	ATCTTACCGC	TGTTGAGATC	CAGTTCGATG	TAACCCACTC	GTGCACCCAA
7451	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG	CGTTTCTGGG	TGAGCAAAAA
7501	CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT
7551	TGAATACTCA	TACTCTTCCT	TTTTCAATAT	TATTGAAGCA	TTTATCAGGG
7601	TTATTGTCTC	ATGAGCGGAT	ACATATTTGA	ATGTATTTAG	AAAAATAAAC
7651	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	AAGTGCCACC	TGACGTCGAC
7701	GGATCGGGAG	ATCTGCTAGG	TGACCTGAGG	CGCGCCGGCT	TCGAATAGCC
7751	AGAGTAACCT	TTTTTTTTAA	TTTTATTTTA	TTTTATTTTT	GAGATGGAGT
7801	TTGGCGCCGA	TCTCCCGATC	CCCTATGGTC	GACTCTCAGT	ACAATCTGCT
7851	CTGATGCCGC	ATAGTTAAGC	CAGTATCTGC	TCCCTGCTTG	TGTGTTGGAG
7901	GTCGCTGAGT	AGTGCGCGAG	CAAAATTTAA	GCTACAACAA	GGCAAGGCTT
7951	GACCGACAAT	TGCATGAAGA	ATCTGCTTAG	GGTTAGGCGT	TTTGCGCTGC
8001	TTCGCGATGT	ACGGGCCAGA	TATACGCGTT	GACATTGATT	ATTGACTAGT
8051	TATTAATAGT	AATCAATTAC	GGGGTCATTA	GTTCATAGCC	CATATATGGA
8101	GTTCCGCGTT	ACATAACTTA	CGGTAAATGG	CCCGCCTGGC	TGACCGCCCA
8151	ACGACCCCCG	CCCATTGACG	TCAATAATGA	CGTATGTTCC	CATAGTAACG
8201	CCAATAGGGA	CTTTCATTG	ACGTCAATGG	GTGGACTATT	TACGGTAAAC
8251	TGCCCCACTTG	GCAGTACATC	AAGTGTATCA	TATGCCAAGT	ACGCCCCCTA
8301	TTGACGTCAA	TGACGGTAAA	TGGCCCGCCT	GGCATTATGC	CCAGTACATG
8351	ACCTTATGGG	ACTTTCCTAC	TTGGCAGTAC	ATCTACGTAT	TAGTCATCGC
8401	TATTACCATG	GTGATGCGGT	TTTGGCAGTA	CATCAATGGG	CGTGGATAGC
8451	GGTTTGACTC	ACGGGGATTT	CCAAGTCTCC	ACCCCATGGA	CGTCAATGGG
8501	AGTTTGTTTT	GGCACCAAAA	TCAACGGGAC	TTTCCAAAAT	GTCGTAACAA
8551	CTCCGCCCCA	TTGACGCAAA	TGGGCGGTAG	GCGTGTACGG	TGGGAGGTCT
8601	ATATAAGCAG	AGCTCTCTGG	CTAACTAGAG	AACCCACTGC	TTACTGGCTT
8651	ATCGAAATTA	ATACGACTCA	CTATAGGGAG	ACCCAAGCTT	

FIG. 18F

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FIG. 19A

-IG. 19A						pD17-hG1b																																																					
10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600
GGGTACCAATT CCATGGTTAA	TAAATTGATA ATTTAACTAT	TCTCCTTAGG AGAGGAATCC	TCTCGAGTCT AGAGCTCAGA	CTAGATAACC GATCTATTGG	GGTCAATCGA CCAGTTAGCT	TTTGGAATTCT AACCTTAAGA	TGCGGCCGCT ACGCCGGCGA	TGCTAGCAC ACGATCGTGG	AAGGSCCAT TTCCCGGTA	CGGTCCTCCC GCCAGAAGGG	CCTGGCACCC GGACCGTGGG	TCCTCCAAGA AGGAGGTTCT	GCACCTCTGG CGTGGAGACC	GGGCACAGCG CCCGTGTCCG	GCGCAGCCGA CGGACCCCGA	GCCTGGTCAA CGGACCAAGT	GGACTACTTC CCTGATGAAG	CCCCGAACCGG GGGCTTGGCC	TGACGGTGTG ACTGCCACAG	GTGGAACTCA CACCTTGAGT	GGCGCCCTGA CCGCGGGACT	CCAGCGGGGT GGTCGCCGCA	GCACACCTTC CGTGTGGAAG	CCGGCTGTCC GGCCGACAGG	CAGAGTCTTC ATGTCAGGAG	TACAGTCTTC CGTGGGTCTG	GCACCCAGAC CGTGGGTCTG	CTACATCTGC GATGTAGACG	AACGTGAATC TTGCACTTAG	ACNAGCCCAG TGTTCCGGTC	CAACACCAAG GTTGTGGTTC	GTGGACAAGA CACCTGTTCT	AGAGTCTGGA TTCAACCACT	GAGGCCAGCA CTCCGGTCTG	GGGTGTCTGC CCCACAGACG	TGGAAGCCAG ACCTTCGGTC	GCTCAGCGCT CGAGTCGCGA	GCAGCAAGGC CGTCGTTCCG	AGGCCCGTCC TCAGGGAGAG	AGGCCCCGTC TCCGGGGCAG	GGTCAATCGC GGTGAGTACG	TCAGGGAGAG AGTCCCTCTC	GGTCTTCTGG CCAGAAAGGG	CTATTTTCCC GAAAAGGGG	CTAGGTGCCC GATCCACGGG	CTAACCCAGG GATTGGGTCC													

FIG. 19B

pD17-hG1b									
610	620	630	640	650	660				
CCCTGCACAC	AAAGGGCAG	GTGCTGGGCT	CAGACCTGCC	AAAGCCATA	TCCGGGAGGA				
GGGACGTGTG	TTTCCCGTC	CACGACCCGA	GTCTGGACGG	TTCTCGGTAT	AGGCCCTCCT				
670	680	690	700	710	720				
CCCTGCCCCCT	GACCTAAGCC	CACCCCAAAG	GCCAAACTCT	CCACTCCCTC	AGCTCGGACA				
GGGACGGGGA	CTGGATTGG	GTGGGGTTTC	CGGTTTGAGA	GGTGAGGAG	TCGAGCCTGT				
730	740	750	760	770	780				
CCTTCTCTCC	TCCAGATTTC	CAGTAACCTC	CAATCTTCTC	TCTGCAGAGC	CCAAATCTTG				
GGAAAGAGAG	AGGCTCTAAG	GTCAATTGAG	GTAGAAGAG	AGACGTCTCG	GGTTTAGAAC				
790	800	810	820	830	840				
TGACAAAAC	CACACATGCC	CACCGTGCCC	AGGTAAGCCA	GCCCAGGCCT	CGCCCTCCAG				
ACTGTTTTGA	GTGTGTACGG	GTGGCACGGG	TCCATTTCGGT	CGGCTCCGGA	GCGGGAGGTC				
850	860	870	880	890	900				
CTCAAGGCGG	GACAGGTGCC	CTAGAGTAGC	CTGCATCCAG	GGACAGGCC	CAGCCGGGTG				
GAGTTCGGCC	CTGTCCACGG	GATCTCATCG	GACGTAGGTC	CCTGTCCGGG	GTCGGGCCAC				
910	920	930	940	235	960				
CTGACACGTC	CACCTCCATC	TCTTCTCTCAG	CACCTGAACT	CCTGGGGGGA	CCGTCACTCT				
GACTGTGCAG	GTGGAGGTAG	AGAAGGAGTC	GTGGACTTGA	GGACCCCCCT	GGCAGTCAGA				
970	980	990	1000	1010	1020				
TCCTCTTCCC	CCCCAAACCC	AAGGACACCC	TCATGATCTC	CCGGACCCCT	GAGGTACAT				
AGGAGAAGGG	GGGTTTGGG	TTCTCTGTGG	AGTACTAGAG	GGCTGGGGA	CTCCAGTGTA				
1030	1040	1050	1060	1070	1080				
GCGTGTGGT	GGACGTGAGC	CACGAAGACC	CTGAGGTCAA	GTTCAACTGG	TACGTGGACG				
CGCACCAACA	CCTGCACCTG	GTGCTTCTGG	GACTCCAGTT	CAAGTTGACC	ATGCACCTGC				
1090	1100	1110	1120	1130	1140				
GCGTGGAGGT	GCATAATGCC	AAGACAAAGC	CGCGGGAGGA	GCAGTACAAC	AGCACGTACC				
CGCACCTCCA	CGTATTACGG	TTCTGTGTTTCG	GCGCCCTCCT	CGTCATGTTG	TCGTGCATGG				
1150	1160	1170	1180	1190	1200				
GTGTGGTCAG	CGTCCCTCACC	GTCCTGCACC	AGGACTGGCT	GAATGGCAAG	GAGTACAAGT				
CACACCAGTC	GCAGGAGTGG	CAGGACGTGG	TCCTGACCGA	CTTACCGTTC	CTCATGTTCA				

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FIG. 19C

				pD17-hG1b			
322	1210	1220	1230331	1240	1250	1260	
GCAAGGCTC	CAACAAAGCC	CTCCACGCC	CCATCGAGAA	AACCATCTCC	AAAGCCAAAG		
CGTTCCAGAG	GTTGTTTCGG	GAGGGTCGGG	GCTAGCTCTT	TTGGTAGAGG	TTTCGGTTTC		
1270	1280	1290	1300	1310	1320		
GTGGGACCG	TGGGGTGCGA	GGGCCACATG	GACAGAGGCC	GGCTCGGCC	ACCTCTTGCC		
CACCTTGGC	ACCCACGCT	CCCGGTGTAC	CTGTCTCCGG	CCGAGCCGGG	TGGGAGACGG		
1330	1340	1350	1360	1370	1380		
CTGAGAGTGA	CCGCTGTACC	AACCTCTGTC	CCTACAGGGC	AGCCCCGAGA	ACCACAGGTG		
GACTCTCACT	GGCGACATGG	TTGGAGACAG	GGATGTCCCG	TCCGGGCTCT	TGGTGTCCAC		
1390	1400	1410	1420	1430	1440		
TACACCCTGC	CCCCATCCCG	GGATGAGCTG	ACCAAGAACC	AGGTCAGCCT	GACCTGCCTG		
ATGTGGGACG	GGGGTAGGGC	CCTACTCGAC	TGGTCTTGG	TCCAGTCGGA	CTGGACGGAC		
1450	1460	1470	1480	1490	1500		
GTCAAAGGCT	TCTATCCAG	CGACATCGCC	GTGGAGTGGG	AGAGCAATGG	GCAGCCGGAG		
CAGTTTCCGA	AGATAGGGTC	GCTGTAGCGG	CACCTCACCC	TCTCGTTACC	CGTCGGCCTC		
1510	1520	1530	1540	1550	1560		
AACAACTACA	AGACCAGGCC	TCCCGTGCTG	GACTCCGACG	GCTCCTTCTT	CCTCTACAGC		
TTGTTGATGT	TCTGGTGGCG	AGGGCACGAC	CTGAGGCTGC	CGAGGAAGAA	GGAGATGTCTG		
1570	1580	1590	1600	1610	1620		
AAGCTCACCG	TGGACAAGAG	CAGGTGGCAG	CAGGGGAACG	TCTTCTCATG	CTCCGTGATG		
TTCCAGTGGC	ACCTGTTCTC	GTCCACCCGTC	GTCCCCCTTGC	AGAAAGAGTAC	GAGGCACCTAC		
1630	1640	1650	1660	1670	1680		
CATGAGGCTC	TGCACAACCA	CTACACGCGAG	AAGGCCTCT	CCCTGTCTCC	GGGTAAATGA		
GTACTCCGAG	ACGTGTTGGT	GATGTGCGTC	TTCTCGGAGA	GGGACAGAGG	CCCATTTACT		
1690	1700	1710	1720	1730	1740		
GTGCGACGGC	CGGCAAGCCC	CCGCTCCCCG	GGCTCTCGCG	GTCCGACCGAG	GATGCTTGGC		
CACGCTGCCG	GCCGTTCCGG	GGCGAGGGGC	CCGAGAGCGC	CAGCGTGCTC	CTACGAACCG		
1750	1760	1770	1780	1790	1800		
ACGTACCCCC	TGTACATACT	TCCCGGGCGC	CCAGCATGGA	AATAAGCAC	CCAGCGCTGC		
TGCATGGGGG	ACATGTATGA	AGGGCCCGCG	GGTCGTACCT	TTATTTCCGTG	GGTCGGGACG		

pD17-hG1b

1860	CTGAGGCTG	1850	CAGGCGAGT	1840	TTCCACGGT	1830	TGATGGTTCT	1820	TGCGAGCTG	1810	CCTGGGCCCC
	GACTCCGGAC		GTCCGGCTCA		AAGGTGCCA		ACTACCAAG		ACGCTCTGAC		GGACCCGGGG
1920	GCTGTGCAGG	1910	ACTGGCCAG	1900	CTGTCCCCAC	1890	GCGGTCCCA	1880	GGGAGGCAGA	1870	AGTGGCATGA
	CGACACGTCC		TGACCGGGTC		GACAGGGGTG		CGCCCAGGT		CCCTCCGTCT		TCACCGTACT
1980	GGTGGGGGAT	1970	CCCTCGGCAG	1960	CCAGGGGCTG	1950	TGGGGCTCAG	1940	CCCCCTAGGG	1930	TGTGCTGGG
	CCACCCCCCTA		GGGAGCCGTC		GGTCCCCGAC		ACCCCGAGTC		GGGGGATCCC		ACACGGACCC
2040	GGAAGCCCTA	2030	CTGGGCCACG	2020	CTGCCCTGGG	2010	CCAGCAGCAC	2000	GGCCCTCCCT	1990	TTGCCAGCGT
	CCTTCGGGAT		GACCCCGGTG		GACGGGACCC		GGTGTCTGTG		CCGGGAGGGA		ACCGTCTGCA
2100	TGTTCTGTGA	2090	GAGACTGTCC	2080	GCCTCTGTAG	2070	CACAGCCCT	2060	GGGACAGACA	2050	GGAGCCCCCTG
	ACAAGACACT		CTCTGACAGG		CGGAGACATC		GTGTGCGGGA		CCCTGTCTGT		CCTCGGGGAC
2160	GCGGTGGGCT	2150	TGCTGGGGAT	2140	CTCGGGGCA	2130	TCCATGCCCC	2120	CCTCCCGACC	2110	GCGCCCTGT
	CGCCACCCGA		ACGACCCCTA		GAGCCCCGT		AGGTACGGGT		GGAGGGCTGG		CGCGGGGACA
2220	CACGCGCCCT	2210	GGGGTATCC	2200	GGGGCTCTAG	2190	AGAACAGCT	2180	TGAGGCGGA	2170	CTATGGCTTC
	GTGCGCGGGA		CCCCATAGGG		CCCCGAGATC		TCTTGTGGA		ACTCGCCTT		GATACCGAAG
2280	GCTACACTTG	2270	CAGCGTGACC	2260	TGTTTAGCGG	2250	GCGGGTGTGG	2240	ATTAAAGCGG	2230	GTAGCGGGCG
	CGATGTGAAC		GTGCGACTGG		ACCAATGCGC		CGCCACACC		TAATTGCGC		CATCGCCGCG
2340	ACGTTCCGCG	2330	CTTCTCGCC	2320	TCTTCCCTTC	2310	CCTTTCGCTT	2300	AGCGCCGCT	2290	CCAGCGCCCT
	TGCAAGCGCG		GAAAGAGCGG		AGAAGGGAAG		GGAAAGCGAA		TGCGGGGCGA		GGTGGCGGGA
2400	AGTGCCTTAC	2390	GTTCGGATT	2380	TCCCTTTAGG	2370	AATCGGGCA	2360	TCAAGCTCTA	2350	GCTTTCCTCG
	TCACGAAATG		CAAGGCTAAA		AGGGAATCC		TTAGCCCCGT		AGTTCAGAT		GBAAAGGGGC



FIG. 19E

2410	2420	2430	2440	2450	2460
GGGACCTCGA	CCCCAAAA	CTTGATTAGG	GTGATGGTTC	ACGTAGTGGG	CCATCGCCTT
CCGTGGAGCT	GGGGTTTTTT	GAACATAATCC	CACTACCAAG	TGCATCACCC	GGTAGCGGGA
2470	2480	2490	2500	2510	2520
GATAGACGGT	TTTTCGCCCT	TTGACGTTGG	AGTCCACGTT	CTTTAATAGT	GGACTCTTGT
CTATCTGCCA	AAAAGCGGGA	AAC TGCAACC	TCAGGTGCAA	GAATATATCA	CCTGAGAACA
2530	2540	2550	2560	2570	2580
TCCAAACTGG	AACAACACTC	AAC CCTATCT	CGGTCTATTCT	TTTTGATTTA	TAAGGGAATTT
AGGTTTGACC	TTGTTGTGAG	TTGGGATAGA	GCCAGATAAG	AAAACTAAAT	ATTCCCTAAA
2590	2600	2610	2620	2630	2640
TGGGGATTTC	GGCCTATTGG	TTAAAAAATG	AGCTGATTTA	ACAAAAATTT	AACGGGAATTT
ACCCCTAAAG	CCGGATNACC	AATTTTTTAC	TCGACTAAAT	TGTTTTTAAA	TTGCGCTTAA
2650	2660	2670	2680	2690	2700
AAATCTGTGG	AATGTGTGTC	AGTTAGGGTG	TGGAAGTGCC	CCAGGCTCCC	CAGGCAGGCA
TTAAGACACC	TTACACACAG	TCAATCCCAC	ACCTTTCAGG	GGTCCGAGGG	GTCCGTCCGT
2710	2720	2730	2740	2750	2760
GNAGTATGCA	AAGCATGCAI	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	CTAACTCCGC
CTTCATACGT	TTCGTACGTA	GAGTTAATCA	GTCGTTGGTA	TCAGGGCGGG	GATTGAGGGG
2770	2780	2790	2800	2810	2820
CCATCCCGCC	CCTAACTCCG	CCCAGTTCGG	CCCATTCTCC	GCCCCATGGC	TGACTAAATTT
GGTAGGGCGG	GGATTGAGGC	GGGTCAAGGC	GGGTAAAGAG	CGGGGTACCG	ACTGATTTAA
2830	2840	2850	2860	2870	2880
TTTTTATTTA	TGCAGAGGCC	GAGGCGGCCT	CGGCCTCTGA	GCTATTTCCAG	AAGTAGTGAG
AAAAATAAAT	ACGTCTCCCG	CTCCGGCGGA	GCCGGAGACT	CGATAAGGTC	TTCATCACTC
2890	2900	2910	2920	2930	2940
GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	GCTGCCGATTT
CTCCGNAAAA	ACCTCCGGAT	CCGNAAAACGT	TTTTTCGAACC	TGTCGAGTCC	CGACGCTAAA
2950	2960	2970	2980	2990	3000
CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	CCCGCTGCCA
GGCGCGTTTG	AACTGCCGTT	AGGATCGCAC	TTCCGACCAT	CCTAAATAAG	GGCGGACGGT

FIG. 19F		pD17-hG1b	
3010	3020	3030	3040
TCATGGTTTCG	ACCATTTGAC	TGCATCGTCG	CCGTGTCCCA
AGTACCAAGC	TGGTAACTTG	ACGTAGCAGC	GGCACAGGGT
3070	3080	3090	3100
ACGGAGACCT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA
TGCCTCTGGA	TGGGACCGGA	GGCGAGTCTT	TGCTCAAGTT
3130	3140	3150	3160
CAACCTCTTC	AGTGGAGGTT	AAACAGAATC	TGGTGATTAT
GTTGGAGNAG	TCACCTTCCA	TTTGTCTTAG	ACCACCTAATA
3190	3200	3210	3220
CCATTCTCTGA	GAAAGATCGA	CCTTTAAAGG	ACAGAAATTAA
GGTAAGGACT	CTTCTTAGCT	GGAAATTTCC	TGTCTTAATT
3250	3260	3270	3280
TCAAAGAAC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG
AGTTTCTTGG	TGGTCTCTCT	CGAGTAAAG	AACGGTTTTC
3310	3320	3330	3340
TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT
AATAACTTGT	TGGCCTTAAC	CGTTCAATTC	ATCTGTACCA
3370	3380	3390	3400
CTGTTTACCA	GGAGGCCATG	AATCAACCCAG	GCCACCTTAG
GACAAATGGT	CCTTCGGTAC	TTAGTTGGTC	CGGTGGAATC
3430	3440	3450	3460
TGCAGGAATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAAATTGA
ACGTCCTTAA	ACTTTCACCTG	TGCNAAAAGG	GTCTTTAACT
3490	3500	3510	3520
TCCCAGATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA
AGGGTCTTAT	GGGTCCGCAG	GAGAGACTCC	AGGTCTCTCT
3550	3560	3570	3580
TTGAAGTCTA	CGAGAGAGAA	GACTAACAGG	AAGATGCTTT
AACCTCAGAT	GCTCTTCTTT	CTGATTTGTC	TTCTACGAAA
3600	3610	3620	3630
GCTCCCTCTCC	CGAGGGGAGG	CGAGGGGAGG	CGAGGGGAGG



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FIG. 19G		pD17-hG1b	
3610	TAAAGCTATG	3630	AGACCATGGG
	ATTTCGATAC	3640	ACTTTTGCTG
			TGAAACGAC
3670	GGAACCTTAC	3690	TGACATAATT
	CCTTGGAATG	3700	GGACAACTA
			CCTGTTTGAT
3730	TAAGGTAANT	3750	TAAGTGATATA
	ATTCCATTTA	3760	ATGTGTTAAA
			TACACAATTT
3790	TGTATTTTAG	3810	ATGGAACCTG
	ACATAAAATC	3820	TGAATGGGAG
			ACTTACCCTC
3850	TGAGGAAAC	3870	CAGAAGAAAT
	ACTCCTTTTG	3880	GCCATCTAGT
			CGGTAGATCA
3910	CTCTCAACAT	3930	CAAAAGAGAA
	GAGAGTTGTA	3940	GAGAAAGGTA
			CTCTTTCCAT
3970	TTCAGAAATG	3990	TGAGTCATGC
	AAGTCTTAAC	4000	ACTCAGTACG
			ACACAAATCA
4030	TGCTATTATC	4050	AAAAAGCTGC
	ACGATAAATG	4060	TTTTTCGACG
			TGGTGTTTCC
4090	TTCTGTAAAC	4110	GGCATAACAG
	AAGACATTGG	4120	TTATAATCAT
			CGGTATTGTC
4150	TCCACACAGG	4170	CTGCTATTAA
	AGGTGTGTCC	4180	TAAGTATGCT
			ATTGATACGA
		4190	CAAAATTTGT
		4200	GTACCTTTAG
			CATGGAAATC
			GTACCTTTAG
			CATGGAAATC

pD17-hG1b

4210	CTTTTAAAT	4220	TTAATAAGGA	4230	ATATTGATG	4240	TATAGTGCT	4250	TGACTAGAGA
	GAAAAATTA	4220	AAATATTCT	4230	TATAAACTAC	4240	ATATCAGGA	4250	ACTGATCTCT
		4220	TTTGTAGAG	4230	TTTACTTGC	4240		4250	
		4220	AAACATCTCC	4230	AAAATGAACG	4240		4250	
		4220		4230		4240		4250	
4270	TCATAATCAG	4280	TTTGTAGAG	4290	TTTACTTGC	4300	TTTAAAAAAC	4310	CTCCACACC
	AGTATTAGTC	4280	AAACATCTCC	4290	AAAATGAACG	4300	AAATTTTTTG	4310	GAGGTTGTGG
		4280	AAAATGAATG	4290	CAATTGTTGT	4300		4310	
		4280	TTTACTTAC	4290	GTTAACAAAC	4300		4310	
		4280		4290		4300		4310	
4330	TCCCCCTGAA	4340	AGCAATAGCA	4350	TCACAAATTT	4360	TGTTAACTTG	4370	TTTATTGCAG
	AGGGGGACTT	4340	TCGTATCGT	4350	AGTGTTTAAA	4360	ACAAATTGAAC	4370	AAATAACGTC
		4340		4350		4360		4370	
		4340		4350		4360		4370	
		4340		4350		4360		4370	
4390	CTTATAATGG	4400	AGCAATAGCA	4410	TCACAAATTT	4420	CACAAATAAA	4430	GCATTTTTTT
	GAATATTACC	4400	TCGTATCGT	4410	AGTGTTTAAA	4420	GTGTTTATTT	4430	CGTAAAAAAA
		4400		4410		4420		4430	
		4400		4410		4420		4430	
		4400		4410		4420		4430	
4450	CACCTGCATT	4460	TTGTCCAAAC	4470	TCATCAATGT	4480	ATCTTATCAT	4490	GTCTGGATCG
	GTGACGTAAG	4460	AACAGGTTTG	4470	AGTAGTTACA	4480	TAGAATAGTA	4490	CAGACCTAGC
		4460		4470		4480		4490	
		4460		4470		4480		4490	
		4460		4470		4480		4490	
4510	GCTGGATGAT	4520	GGGGATCTCA	4530	TGCTGGAGTT	4540	CTTCGCCAC	4550	CCCAACTTGT
	CGACCTACTA	4520	CCCCTAGAGT	4530	ACGACCTCAA	4540	GAAGCGGGTG	4550	GGGTTGAACA
		4520		4530		4540		4550	
		4520		4530		4540		4550	
		4520		4530		4540		4550	
4570	TTATTGCAGC	4580	TACAAATAAA	4590	GCAATAGCAT	4600	CACAAATTC	4610	ACAAATAAAG
	AATAACGTCG	4580	ATGTTTATTT	4590	CGTTATCGTA	4600	GTGTTTAAAG	4610	TGTTTATTTT
		4580		4590		4600		4610	
		4580		4590		4600		4610	
		4580		4590		4600		4610	
4630	CATTTTTTTC	4640	AGTTGTGGTT	4650	TGTCCAAAC	4660	CATCAATGTA	4670	TCTTATCATG
	GTAANAANAAG	4640	TCAACACCAA	4650	ACAGGTTTGA	4660	GTAGTTACAT	4670	AGAAATAGTAC
		4640		4650		4660		4670	
		4640		4650		4660		4670	
		4640		4650		4660		4670	
4690	TCTGTATACC	4700	AGCTAGAGCT	4710	TGGCGTAATC	4720	ATGGTCATAG	4730	CTGTTTCCCTG
	AGACATATGG	4700	TCGATCTCGA	4710	ACCGCATTAG	4720	TACCAGTATC	4730	GACAAAGGAC
		4700		4710		4720		4730	
		4700		4710		4720		4730	
		4700		4710		4720		4730	
4750	TGTGAATTTG	4760	ACAATTCAC	4770	ACAACATACG	4780	AGCCGGAAGC	4790	ATAAAAGTGA
	ACACTTTAAC	4760	TGTTAAGGTG	4770	TGTTGTATGC	4780	TCGGCCTTCG	4790	TATTTTCACAT
		4760		4770		4780		4790	
		4760		4770		4780		4790	
		4760		4770		4780		4790	



FIG. 191

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4810	4820	4830	4840	4850	4860
AAGCCTGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	TCAGTGCCCG
TTTCGACCCC	ACGGATTACT	CACCTCGATTG	AGTGTAAATTA	ACGCAACGCG	AGTGACGGGC
4870	4880	4890	4900	4910	4920
CTTTCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA
GAAAGGTCAG	CCCTTTGGAC	AGCACGGTCG	ACGTAATTAC	TTAGCCGGTT	CGCGGCCCTT
4930	4940	4950	4960	4970	4980
GAGGCGGTTT	GGGTATTGGG	CGCTCTTCCG	CTTCTCTCGT	CACTGACTCG	CTGCGCTCGG
CTCCGCCAAA	CGCATAACCC	GGGAGAAGGC	GAAAGGACGA	GTGACTGAGC	GACGCGAGCC
4990	5000	5010	5020	5030	5040
TGTTTCGGCT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG
AGCAAGCCGA	CGCCGCTCGC	CATAGTCGAG	TGAGTTTCCG	CCATTATGCC	AATAGGTGTC
5050	5060	5070	5080	5090	5100
AATCAGGGGA	TAAAGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAAAC
TTAGTCCCT	ATTGCGTCCT	TTCTTGTAACA	CTCGTTTTCC	GGTCGTTTTC	CGTCCCTTGG
5110	5120	5130	5140	5150	5160
GTAANAAGGC	CGCGTTGCTG	GGGTTTTTCC	ATAGGCTCCG	CCCCCCTGAC	GAGCATCACAA
CATTTTTCCG	GCGCAACGAC	CGCAAAAAGG	TATCCGAGGC	GGGGGGACTG	CTCGTAGTGT
5170	5180	5190	5200	5210	5220
AAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT
TTTTAGCTGC	GAGTTCAGTC	TCCACCGCTT	TGGGCTGTCC	TGATATTCT	ATGGTCCGCA
5230	5240	5250	5260	5270	5280
TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTPTCCGAC	CCTGCGCGTT	ACCGGATACC
AAGGGGGACC	TTCCAGGGAG	CACGCGAGAG	GACAAGGCTG	GGACGGCGAA	TGGCCTATGG
5290	5300	5310	5320	5330	5340
TGTCGCGCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTTCTCA	ATGCTCAGCG	TGTAGGTATC
ACAGGCGGAA	AGAGGGAAGC	CCTTCGCACC	GCGAAAGAGT	TACGAGTGCG	ACATCCATAG
5350	5360	5370	5380	5390	5400
TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAAACC	CCCGTTACGC
AGTCAAGCCA	CATCCAGCAA	GCGAGGTTCC	ACCCACACA	CGTCTCTGGG	GGGCAAGTCG

FIG. 19J

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5410	5420	5430	5440
CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC
GGCTGGCGAC	GCGGAATAGG	CCATTGATAG	CAGAACTCAG
5470	5480	5490	5500
TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG
ATAGCGGTGA	CCGTGCTCGG	TGACCAATTGT	CCTAATCGTC
5530	5540	5550	5560
CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC
GATGTCTCAA	GAACTTCACC	ACCGGATTGA	TGCCGATGTG
5590	5600	5610	5620
TCTGGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT
AGACGCGAGA	CGACTTCGGT	CAATGGAAGC	CTTTTCTCA
5650	5660	5670	5680
AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA
TTGTTTGGTG	GCGACCATCG	CCACCACAAA	AACAAACGTT
5710	5720	5730	5740
AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG
TTTTTCCTAG	AGTTCTTCTA	GGAATCTAGA	AAAGATGCCC
5770	5780	5790	5800
AAAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA
TTTTGAGTGC	AATTCCCTAA	AACCAGTACT	CTAATAGTTT
5830	5840	5850	5860
TTTTAAATTA	AAATGAAGT	TTTAAATCAA	TCTAAAGTAT
AAATTTAAT	TTTTACTTCA	AAATTTAGTT	AGATTTTCATA
5890	5900	5910	5920
ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC
TGTCAATGGT	TACGAATTAG	TCACCTCCGTG	GATAGAGTCG
5950	5960	5970	5980
CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT
GGTATCAACG	GACTGAGGGG	CAGCATCT	ATTGATGCTA
			5990
			ACGGGAGGGC
			TTACCATCTG
			AATGGTAGAC
			6000
			TTACCATCTG
			AATGGTAGAC

FIG. 19K

				pD17-hG1b			
6010	6020	6030	6040	6050	6060		
GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	TTATCAGCAA		
CGGGGTACAG	ACGTTACTAT	GGCGCTCTGG	GTGCGAGTGG	CCGAGGTCTA	AATAGTCGTT		
6070	6080	6090	6100	6110	6120		
TAAACCAGCC	AGCCGGAGGG	GCCGAGCGCA	GAACTGGTCC	TGCAACTTAA	TCCGCCCTCCA		
ATTGGTCTGG	TCGGCCCTTCC	CGGCTCGCGT	CTTCACCAGG	ACGTTGAAT	AGCGGAGGT		
6130	6140	6150	6160	6170	6180		
TCAGTCTAT	TAATTGTTC	CGGGAAGCTA	GAGTAAGTAG	TTGCGCCAGTT	AATAGTTTGC		
AGGTCAGATA	ATTAACAACG	GCCCTTCGAT	CTCATTTCATC	AAGCGGTCAA	TTATCAACCG		
6190	6200	6210	6220	6230	6240		
GCAACGTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACAG	CTCGTCGTTT	GGTATGGCTT		
CGTTGCAACA	ACGGTAACGA	TGTCCGTAGC	ACCACAGTGC	GAGCAGGCAA	CCATACCGAA		
6250	6260	6270	6280	6290	6300		
CATTAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGTGCAAAA		
GTAAGTCGAG	GCCAAGGGTT	GCTAGTTCCG	CTCAATGTAC	TAGGGGGTAC	AACACGTTTT		
6310	6320	6330	6340	6350	6360		
AAGCGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAAGAAG	TAAGTTGGCC	GCAGTGTAT		
TTCCGCCAATC	GAGGAAGCCA	GGAGGCTAGC	AACAGTCTTC	ATTCAACCCG	CGTCACAAATA		
6370	6380	6390	6400	6410	6420		
CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	GTAAGATGCT		
GTGAGTACCA	ATACCGTCGT	GACGTATTAA	GAGAAATGACA	GTACGGGTAGG	CATTCTACGA		
6430	6440	6450	6460	6470	6480		
TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGCGACCCGA		
AAAGACACTG	ACCACCTCATG	AGTTGGTTCA	GTAAGACTCT	TATCACATAC	GCCGCTGGCT		
6490	6500	6510	6520	6530	6540		
GTTGCTCTTG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAG		
CAACGAGAAC	GGGCCGCAGT	TATGCCCTAT	TATGGCGGG	TGTATCGTCT	TGAAATTTTC		
6550	6560	6570	6580	6590	6600		
TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GAAACTCTC	AAGGATCTTA	CCGCTGTTGA		
ACGAGTAGTA	ACCTTTTGCA	AGAAAGCCCCG	CTTTTGAGAG	TTCTTAGAAT	GGCGACAACT		

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6610	6620	6630	6640	6650	6660
GATCCAGTTC	GATGTAACCC	ACTGGTGCAC	CCAACCTGATC	TTTCAGCATCT	TTTACTTTTCA
CTAGGTCAAG	CTACATTGGG	TGAGCACGTG	GGTTGACTAG	AAGTCGTAGA	AAATGAAGT
6670	6680	6690	6700	6710	6720
CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAG	GGAATAAGGG
GGTCCGCAAG	ACCCACTCGT	TTTTGTCCTT	CCGTTTTACG	GGTTTTTTTC	CCTTATTCCC
6730	6740	6750	6760	6770	6780
CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTTTCA	ATATTATTGA	AGCATTTATC
GCTGTGCCCTT	TACAACCTTAT	GAGTATGAGA	AGGAAAAAGT	TATAATAACT	TCGTAATAAG
6790	6800	6810	6820	6830	6840
AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAATAAG
TCCCAATAAC	AGAGTACTCG	CCATATGTATA	AACTTACATA	AACTTTTTTTA	TTTGTTTTATC
6850	6860	6870	6880	6890	6900
GGGTTCCGCG	CACATTTCCT	CGAAAAGTGC	CACCTGACGT	CGACGGATCG	GGAGATCTGC
CCCAAGGCGC	GTGTAAAGGG	GCCTTTTCAG	GTGGACTGCA	GCTGCCCTAGC	CCTCTAGACG
6910	6920	6930	6940	6950	6960
TAGGTGACCT	GAGGCGCGCC	GGCTTCGAAT	AGCCAGAGTA	ACCTTTTTTTT	TTAATTTTAT
ATCCACTGGA	CTCCGCGCGG	CCGAAGCTTA	TCGGTCTCAT	TGGAAAAAATA	AATTAAAAATA
6970	6980	6990	7000	7010	7020
TTTATTTTAT	TTTTGAGATG	GAGTTTGGCG	CCGATCTCCC	GATCCCTTAT	GGTCGACTCT
AAATAAAATA	AAAACCTCTAC	CTCAAACCCG	GGCTAGAGGG	CTAGGGGATA	CCAGCTGAGA
7030	7040	7050	7060	7070	7080
CAGTACAATC	TGCTCTGATG	CCGCATAGTT	AAGCCAGTAT	CTGCTCCCTG	CTTGTGRTT
GTCAATGTTAG	ACGAGACTAC	GGCGTATCAA	TTCCGTTCATA	GACGAGGGAC	GAACACACAA
7090	7100	7110	7120	7130	7140
GGAGGTCCGT	GAGTAGTGCG	CGAGCAAAAT	TTAAGCTACA	ACAAGGCAAG	GCTTGACCGA
CCTCCAGCGA	CTCATCACGC	GCTCGTTTTTA	AATTCGATGT	TGTTCCGTTT	CGAACTGGCT
7150	7160	7170	7180	7190	7200
CAATTGCATG	AAGAATCTGC	TTAGGGTTAG	GGCTTTTGCG	CTGCTTCGCG	ATGTACGGGC
GTTAACGTAC	TTCTTAGACG	AATCCCAATC	CGCAAAACGC	GACGAGCGGC	TACATGCCCG

FIG. 19M

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7210	7220	7230	7240	7250	7260
CAGATATACG	CGTTGACATT	GATTATTGAC	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC
GTCTATATGC	GCAACTGTAA	CTAATAACTG	ATCAATAATT	ATCATTAGTT	AATGCCCCAG
7270	7280	7290	7300	7310	7320
ATTAGTTTCAT	AGCCCATATA	TGGAGTTCCG	CGTTACATAA	CTTACGGTAA	ATGGCCCGCC
TAATCAAGTA	TCGGGTATAT	ACCTCAAGGC	GCAATGTAAT	GAATGCCATT	TACCGGGCGG
7330	7340	7350	7360	7370	7380
TGGCTGACCG	CCCAACGACC	CCCGCCCAAT	GACGTCATAA	ATGACGTATG	TTCCCATAGT
ACCGACTGGC	GGGTTGCTGG	GGCGCGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA
7390	7400	7410	7420	7430	7440
AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAC	TATTTACGGT	AAACTGCCCA
TTGCGGTTAT	CCCTGAAGG	TAACTGCAGT	TACCCACCTG	ATAAATGCCA	TTTGACGGGT
7450	7460	7470	7480	7490	7500
CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC	CCTATTGACG	TCAATGACGG
GAACCGTCAT	GTAATTCA	TAGTATACGG	TTCATGCGGG	GGATAACTGC	AGTTACTGCC
7510	7520	7530	7540	7550	7560
TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA	CATGACCTTA	TGGGACTTTC	CTACTTGGCA
ATTTACCGGG	CGGACCGTAA	TACGGGTTCAT	GTACTGGAAT	ACCTGAAAG	GATGAACCGT
7570	7580	7590	7600	7610	7620
GTACATCTAC	GTATTAGTCA	TCGCTATTAC	CATGGTGATG	CGGTTTTGGC	AGTACATCAA
CATGTAGATG	CATAATCAGT	AGCGATAATG	GTACCACCTAC	GCCAAACCCG	TCATGTAGTT
7630	7640	7650	7660	7670	7680
TGGGCGTGGA	TAGCGGTTTG	ACTCACGGGG	ATTTCCAGT	CTCCACCCCA	TTGACGGTCAA
ACCCGCACCT	ATCGCCAAAC	TGAGTGCCCC	TAAAGGTTCA	GAGGTGGGGT	AACTGCAGTT
7690	7700	7710	7720	7730	7740
TGGGAGTTTG	TTTTGGCACC	AAAATCAACG	GGACTTTCCA	AAATGTCGTA	ACRACTCCGC
ACCCCTCAAAC	AAAACCGTGG	TTTTAGTTGC	CCTGAAAGGT	TTTACAGCAT	TGTTGAGGCG
7750	7760	7770	7780	7790	7800
CCCATTGACG	CAAAATGGCG	GTAGGCGTGT	ACGGTGGGAG	GTCTATATAA	GCAGAGCTCT
GGGTAACTGC	GTTTACCCCG	CATCCGCACA	TGCCACCTTC	CAGATATATT	CGTCTCGAGA

FIG. 19N

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7810	7820	7830	7840	7850	7860
CTGGCTAACT	AGAGAACCCA	CTGCTTACTG	GCTTATCGAA	ATTAATACGA	CTCACTATAG
GACCGATTGA	TCTCTTGGGT	GACGAATGAC	CGAATAGCTT	TAATTATGCT	GAGTGATATC
7870	7880				
GGAGACCCAA	GCTT				
CCTCTGGGTT	CGAA				

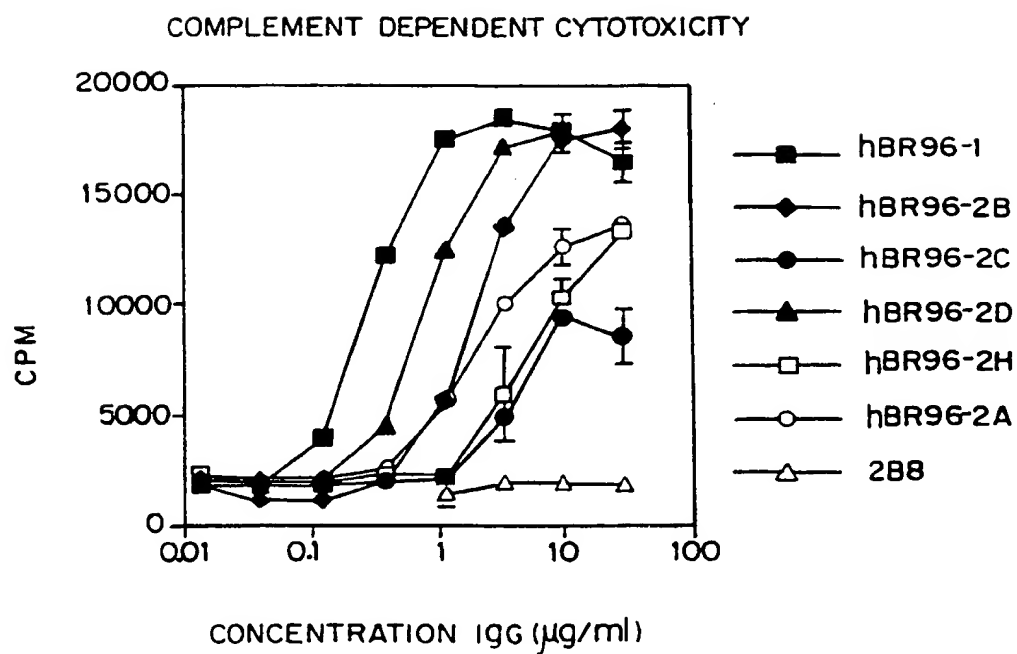
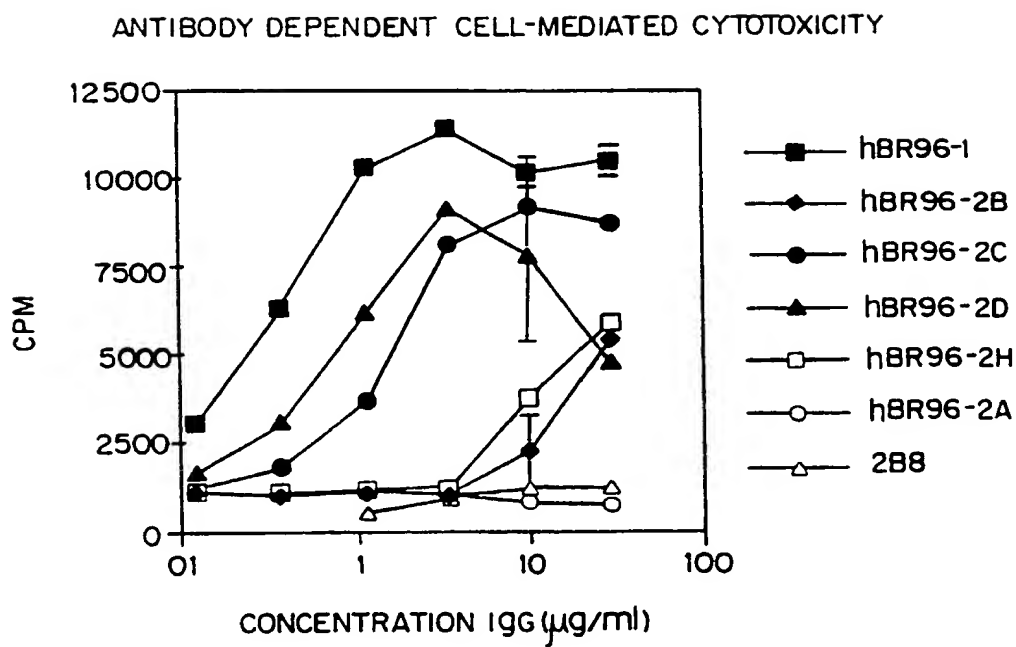
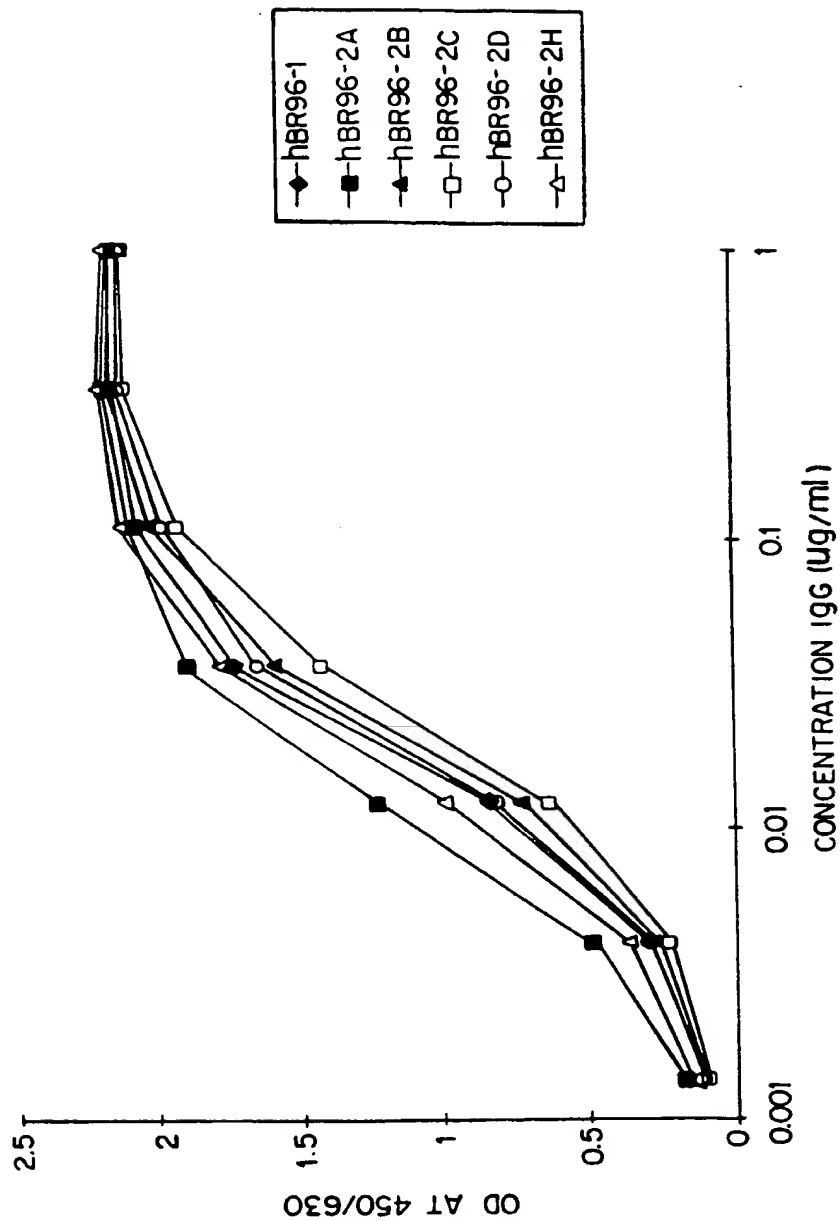
Fig. 20

Fig. 21

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Fig. 22

BINDING ACTIVITY OF hBR96-2 CONSTANT REGION MUTANTS ON LEY-HSA

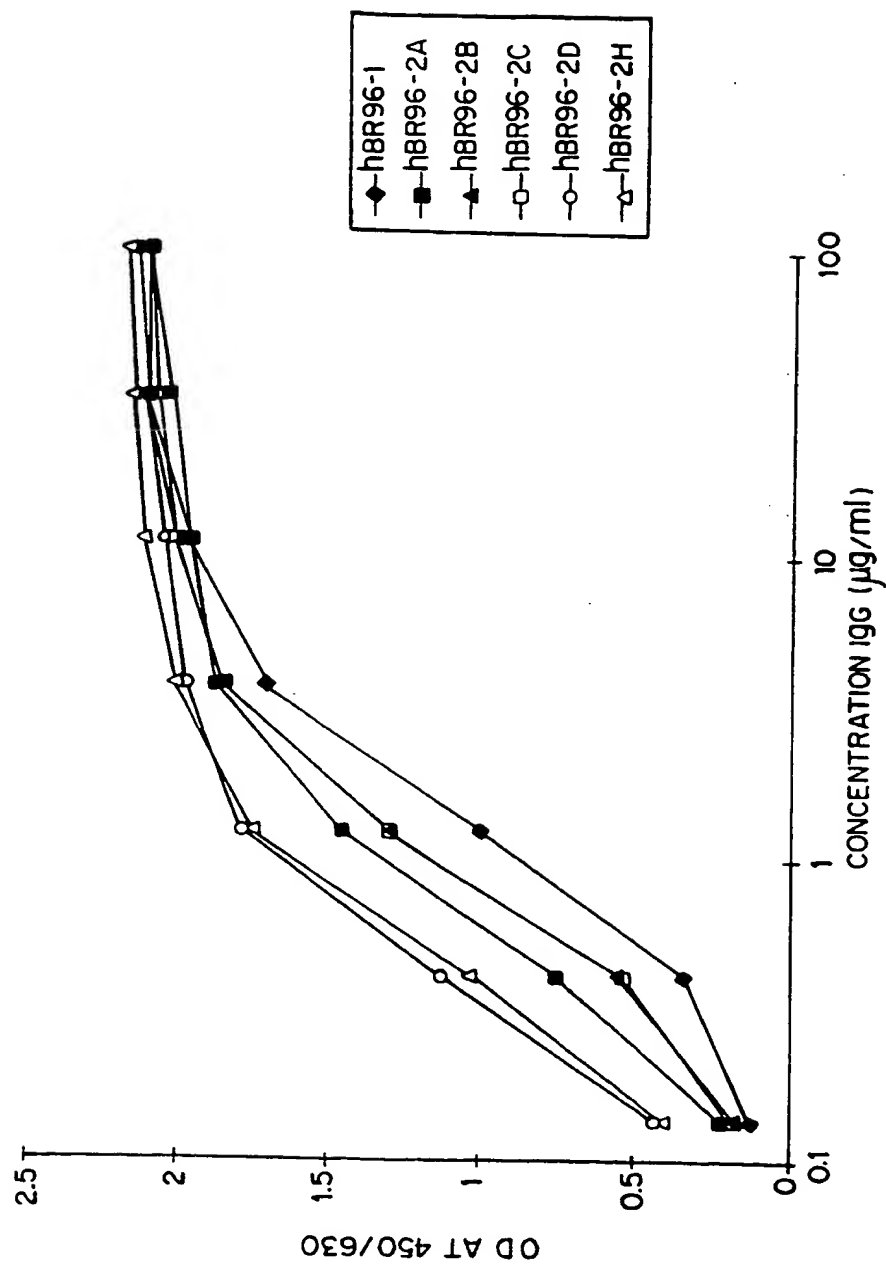


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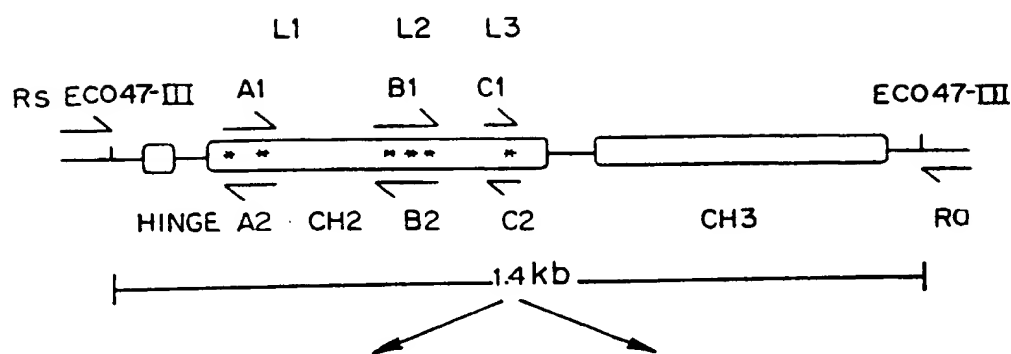
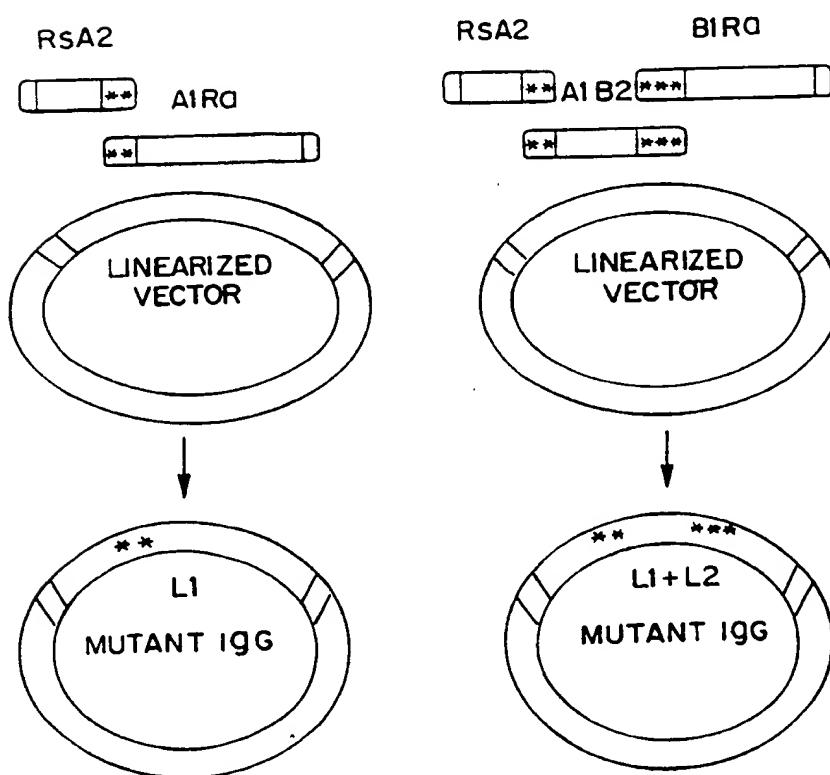
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Fig. 23

BINDING ACTIVITY OF hBR96-2 CONSTANT REGION MUTANTS ON LNFPIII-BSA



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Fig. 24A**Fig. 24B**

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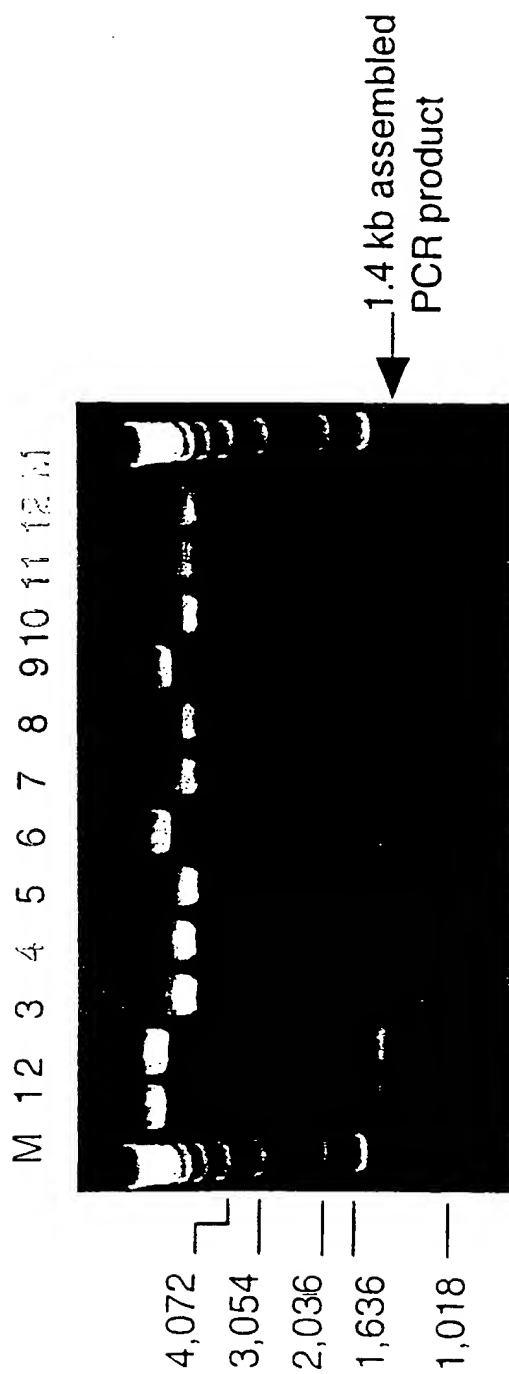


FIG. 25

FIG. 26

hBR96-2 Heavy Chain Variable Region (V_H)

1 11 21 31 41
EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYYMYWVRQA PGKGLEWVSY
51 61 71 81 91
ISQDGDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL
101 111
ADGAWFAYWG QGTLVTVSS

Human IgG1 Constant

CH1
A STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSVVTV PSSSLGTQTY
CH2 235 237
ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CHAPPELLGGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
318 320 322 331 CH3
TYRVVSVLTV LHQDWLNGKE YKDKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

FIG. 27**hBR96-2A: Heavy Chain Variable Region (V_H)**

1 11 21 31 41
EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYMYWVRQA PGKGLEWVSY
51 61 71 81 91
ISQDGDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL
101 111
ADGAWFAYWG QGTLVTVSS

hBR96-2A: Human Heavy Chain IgG1 Constant Region ΔCH2

A STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH
TFPAVLQSSG LYSLSVVTV PSSSLGTQTY ICNVNHNKPSN TKVDKKVEPK
SCDKTHTCPP CP GQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA
VEWESNGQPE NNYKTTTPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
HEALHNHYTQ KSLSLSPGK

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FIG. 28

This sequence is the chi BR96 IgG1 with CH2 deleted.

VH
1 EVNLVESGGG LVQPGGSLKV SCVTSGFTFS DYMYWVRQT PEKRLEWVAY
51 ISQGGDITDY PDTVKGRFTI SRDNAKNTLY LQMSRLKSED TAMYVCARGL
CH1
101 DDGAWFAYWG QGTLVTVSVA STKGPSVFPL APSSKSTSGG TAALGCLVKD
151 YFPEPVTVSW NSGALTSGVH TFAVLQSSG LYSLSVVTV PSSSLGTQTY
CH3
201 ICNVNHNKPSN TKVDKKVEPK SCDKTHTCPP CPGQPREPOV YTLPPSRDEL
251 TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS
301 KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 97/13562

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/62 A61K39/395 A61K38/17 A61K47/48 A61K51/10
C07K16/30 C07K16/46 C07K16/00 C12N15/13 C12N1/21
C12N5/10 //C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. GILLIES ET AL.: "Antigen binding and biological activities of engineered mutant chimeric antibodies with human tumor specificities." HUMAN ANTIBODIES AND HYBRIDOMAS, vol. 1, no. 1, 1990, STONEHAM, MA, USA, pages 47-54, XP002050448 see the whole document --- -/-	1-8, 23-25

☒ Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search

17 December 1997

Date of mailing of the international search report

21. 01. 98

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INTERNATIONAL SEARCH REPORT

1st Application No

PCT/US 97/13562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. SCHREIBER ET AL.: "An unmodified anticarcinoma antibody, BR96, localizes to and inhibits the outgrowth of human tumors in nude mice." CANCER RESEARCH, vol. 52, no. 12, 15 June 1992, BALTIMORE, MD, USA, pages 3262-3266, XP002050449 see abstract	33,35,36
A	---	1,2,5,7, 8,11-18, 23
A	A. DUNCAN ET AL.: "The binding site for Clq on IgG." NATURE, vol. 332, no. 6166, 21 April 1988, LONDON, GB, pages 738-740, XP002050450 cited in the application see the whole document	1,2,5,7, 8
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A	J. LUND ET AL.: "Human Fcγ ₁ RI and Fcγ ₁ RII interact with distinct but overlapping sites on human IgG." THE JOURNAL OF IMMUNOLOGY, vol. 147, no. 8, 15 October 1991, BALTIMORE, MD, USA, pages 2657-2662, XP002050451 cited in the application see abstract	1,2,5,7, 8
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A	Y. XU ET AL.: "Residue at position 331 in the IgG1 and IgG4 CH2 domains contributes to their differential ability to bind and activate complement." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 5, 4 February 1994, BALTIMORE, MD, USA, pages 3469-3474, XP002050452 cited in the application see abstract see discussion	1-8
A	---	1,2,5,7, 8
A	T. MICHAELSEN ET AL.: "One disulfide bond in front of the second heavy chain constant region is necessary and sufficient for effector functions of human IgG3 without a genetic hinge." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 91, no. 20, 27 September 1994, WASHINGTON, DC, USA, pages 9243-9247, XP002050453 see the whole document	1,2,5,7, 8

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 97/13562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	L. TAN ET AL.: "Influence of the hinge region on complement activation, Clq binding, and segmental flexibility in chimeric human immunoglobulins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 87, no. 1, January 1990, WASHINGTON, DC, USA, pages 162-166, XP002050454 see the whole document ---	1-8
A	EP 0 699 756 A (BRISTOL-MYERS SQUIBB COMPANY) 6 March 1996 cited in the application see examples see claims -----	11-18, 23,25, 28,29, 31-52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/13562

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/13562

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 26,27

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 26 represents a method of detection/diagnosis and refers forward to claim 30, which represents a method of treatment. Claim 27 refers to a method in claim 24; however, in claim 24 a product is claimed, not a method.

Remark : Although claims 1-22, 25, 28-32 and 34-36 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

Information on patent family members

PCT/US 97/13562

Form PCT/ISA/210 (patent family annex) (July 1992)

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